APPLICATION OF NUTRIENT ENRICHMENT BIOASSAYS TO EVALUATE SPATIAL AND TEMPORAL LIMITING-NUTRIENT PATTERNS AND TO ESTIMATE SURPLUS PHOSPHORUS CONCENTRATIONS IN LAKE OKEECHOBEE, FLORIDA

BY

FREDERICK JAMES ALDRIDGE

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I dedicate this dissertation to my mother, Harriett Aldridge, for having faith in me and providing me with the resolve to succeed.

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Abstract of Dissertation Presented to the Graduate School
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by

Frederick James Aldridge

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Chairman: Claire Schelske
Cochairman: Edward Phlips
Major Department: Fisheries and Aquatic Sciences

Nutrient enrichment bioassays (NEB) were used to assess the spatial and temporal distribution of nitrogen (N) and phosphorus (P) limitation of phytoplankton biomass and to estimate surplus P in Lake Okeechobee, Florida. Results of three years (1990-92) of monthly assays on samples collected from five stations showed that N was the dominant limiting nutrient. The second most common result was a lack of limitation by either N or P indicating that there was a surplus of both nutrients available for phytoplankton growth. Phosphorus was never a primary limiting nutrient although N and P co-limitation occurred.

Frequency analysis of limiting nutrient classifications showed both spatial and temporal organization to the type and frequency of limiting factors. Limiting nutrient classifications were matched with physical and chemical data to define discriminant functions for N, NP co-limited and nutrient unlimited conditions. Discriminant functions were applied to a larger physical and chemical data set collected from 76 stations over a three-year period to develop maps of spatial and temporal distribution of limiting nutrient classifications. Nitrogen was predicted to be the primary limiting nutrient from the lake shore to the three-meter depth contour for all seasons. In areas deeper than three-meters it was predicted that phytoplankton growth is unlimited by nutrient availability in all but the summer months when N was limiting.

Estimates of surplus P were determined from direct chemical measurements and the phytoplankton biomass (as Chl-*a*) supportable by surplus P was determined using NEB. There was a significant relationship between surplus P and supported Chl-*a*. The average surplus P and supported Chl-*a* at the 5 stations ranged from 13 to 55 µg/L with an overall averages of 31 and 32 µg/L respectively. Both variables were highest at the north and central stations and lowest at the south and southwest stations. Approximately 50% of total P was in the form of surplus P and not expressed as phytoplankton biomass. Quantifying the surplus-P in a lake provides a direct estimate of the amount of P that must be reduced before phytoplankton standing crop will be nutrient limited by P.

CHAPTER 1
INTRODUCTION

The increasing human population in the world has created enormous pressure on environmental resources in general and aquatic resources specifically. This demand on aquatic resources has driven scienctists to study the basic mechanisms of aquatic ecology in order to assess the magnitude of human impact and provide guidance for the intelligent utilization of these limited resources. There, however, is an increasing need for analytical tools that can be used to understand some of the complex interactions in aquatic ecosystems. This dissertation focuses on the utilization of an analytical tool, the nutrient enrichment bioassay (NEB), to identify spatial and temporal distribution of limiting nutrients for phytoplankton and to estimate the surplus phosphorus concentration potentially available for phytoplankton growth.

The impetus for this research was a controversy concerning the trophic status and factors controlling phytoplankton standing crop in Lake Okeechobee, a large shallow lake in south central Florida. One study, based on an empirical nutrient-loading model, concluded Lake Okeechobee is becoming more eutrophic due to anthropogenic loading of phosphorus (P) (Janus et al. 1990). A contrasting study used empirical methods to conclude there was no strong evidence that Lake Okeechobee was changing to a higher trophic state (Canfield and Hoyer 1988). I, therefore, used an experimental approach, NEB, to study phytoplankton-nutrient dynamics in Lake Okeechobee, with the goal of identifying whether nitrogen (N), phosphorus (P) or some other factor controlled phytoplankton biomass in the lake.

The term "nutrient limitation" is often used in different contexts. Howarth (1988) summarized three definitions for nutrient limitation: a) the limitation of growth rate of the phytoplankton community, b) limitation of net ecosystem production and c) limitation of potential net primary production. The last definition, "c," is most applicable to this dissertation when modified to include only phytoplankton. The basis for this definition is Leibig's Law of Minimum (Parsons et al. 1984). It is generally translated as "The yield of a crop will be limited by the essential nutrient that is most scarce in the environment relative to the needs of that crop."

Much research has addressed the question of whether nitrogen (N) or P is the primary nutrient limiting phytoplankton biomass. Redfield (1958) argued that N could not be limiting in the oceans since inorganic N_2 fixation by bacterio-plankton could supply all N requirements. This concept was reinforced in freshwater systems where whole lake experiments on P enrichment indicated P was generally the primary limiting nutrient (Schindler 1977). Ryther and Dunstan (1971), however, disagreed with Redfield and concluded that N was the primary limiting nutrient in coastal oceans. As more studies were made of shallow and tropical freshwater systems N limitation was identified (Henry et al. 1985, Thornton 1987).

Although it is now accepted that it is possible for N to be limiting, it is not possible to conclude that either N or P is universally limiting. Empirical relationships of phytoplankton biomass to N or P, however, are usually based on total concentration of the respective nutrient (Baker et al. 1985, Canfield 1983, Dillon and Rigler 1974). In these relationships, it is implicit that N or P must be clearly limiting such that phytoplankton standing crop is directly related to the total concentration of the limiting nutrient. This implicit assumption is valid only if suspended solids are almost entirely attributed to phytoplankton and most if not all of the total N (TN) and total P (TP) are contained in the phytoplankton (Hoyer and Jones 1983).

In mesotrophic and eutrophic waters or in turbid waters, where phytoplankton do not constitute most of the suspended matter, empirical relationships can be confounded by nonalgal components (Hoyer and Jones 1983) or by biologically unavailable fractions of the nutrients. The stoichiometric relationship of Chl-*a* to TN and TP, that are implied in an empirical model may vary if confounding factors are present (Hoyer and Jones 1983, Phlips et al. In Press-a,b). Under conditions, where TN or TP-chlorophyll relationships are confounded, it would be informative to establish what factor, such as the form of a nutrient, directly controls phytoplankton standing crop.

Some researchers have shown that it is possible to determine N limitation of phytoplankton directly from ambient inorganic N concentrations and/or uptake rates (Healey and Hendzel 1980, Vincent et al. 1984). Stewart et al. (1978) reported a poor correlation between cyanobacteria biomass and levels of dissolved N and P in the water column. Riegman et al. (1990) found that low concentrations of inorganic N and P in the water may not indicate limiting conditions. Rapid growth of phytoplankton has been reported when soluble reactive P was at detection limits (Fuhs et al. 1972, Smith and Kalff 1981). Phytoplankton have been shown to store N and P in the form of phycobiliproteins and polyphosphates, in excess of their immediate metabolic requirements, that can be utilized to support phytoplankton growth when soluble inorganic forms of N and P are low or absent (Fitzgerald and Nelson 1966, Rhee 1972, 1973, 1974, Stewart et al. 1978, Wynne and Berman 1980). Given the complexity of phytoplankton-nutrient dynamics it is understandable why Sommer (1989) concluded that nutrient stoichiometry alone is not adequate, in all cases, to explain temporal and spatial variability in phytoplankton biomass. In order to resolve ambiguity concerning the importantance of N and P in phytoplankton to nutrient relationships it is necessary to assess some dynamic response by phytoplankton to changes in ambient nutrient conditions.

A variety of dynamic response assessments of phytoplankton-nutrient interactions such as measurements of nutrient uptake rates (Lean and White 1983, Rigler 1956), enzymatic activity (Berman 1970, Chrost and Overbeck 1987) and growth responses in NEBs (Dodds and Priscu 1990, Schelske et al. 1978, Schelske 1984, USEPA 1971) have been used to determine the limiting-nutrient status of phytoplankton communities. I chose NEB from these different methods because it has been shown to have fewer interferences and has the least ambiguous interpretation of results (Dodds and Priscu 1990, Elser et al. 1990, Elser and Kimmel 1986, Newman et al. 1994, Peeters and Peperzak 1990).

NEB applies Leibig's Law of Minimum in reverse, in that it tests for what is not limiting. This logic is best described by Gibson's (1971) definition of limitation: a factor is not limiting if an increase in that factor produces no significant stimulation in algal growth. Nutrient enrichment bioassay is the only method that can test for nutrient limitation by more than one nutrient and provides the means to rank the test nutrients by the level of limitation (Peeters and Peperzak 1990).

Nutrient enrichment bioassays can be categorized in two forms: those that assay the nutrient content of filtered lake water by growth response of a laboratory cultured species (Reynolds and Butterwick 1979, USEPA 1971) and those that assay the growth response of the natural phytoplankton community in lake water (Schelske et al. 1974, Schelske 1984). The latter method was chosen for the reason given by Schelske et al. (1978): the natural phytoplankton community is representative of the lake ecosystem and it has a physiological history due to the environmental conditions in the lake. The NEB provides a method to define a limiting nutrient classification which is a qualitative identification of that nutrient which most limits phytoplankton from increasing in biomass.

The objectives of this dissertation were to:

1) Develop qualitative limiting nutrient classifications from NEB of natural phytoplankton assemblages (Chapter 2).
2) Identify spatial and temporal distribution of qualitative limiting-nutrient classifications (Chapter 2).
3) Define discriminant functions relating physical and chemical data to each limiting nutrient classification determined by NEB (Chapter 3).

4) Map the distribution of limiting nutrient classifications, predicted by discriminant functions, by years and seasons (Chapter 3).
5) Use nutrient enrichment bioassays to estimate the quantity of surplus P that can support phytoplankton growth (Chapter 4).
6) Relate supported Chl-*a*, estimated from NEB, to surplus P measured chemically (Chapter 4).
7) Analyze surplus P data for temporal and spatial trends (Chapter 4).

This dissertation is composed of five chapters. Chapter 1 is an introduction. Chapters 2 through 4 are written as sections in preparation for publication. Chapter 5 is the summary. A common list of references is presented at the end of the dissertation.

CHAPTER 2
SPATIAL AND TEMPORAL DIFFERENCES IN LIMITING NUTRIENTS CONTROLLING PHYTOPLANKTON BIOMASS IN LAKE OKEECHOBEE

Introduction

Nutrient availability and phytoplankton community response can be effected by temporal and spatial factors. In a shallow polymictic lake, such as Lake Okeechobee in Florida, temporal variability of phytoplankton biomass may be less related to seasonal cycles in temperature and photoperiod and more related to seasonally controlled stochastic mixing (e.g., wind mixing and storm events) (Ganf 1974, Ganf and Viner 1973, Lewis 1973, Maceina and Soballe 1990). The effect of wind mixing on phytoplankton-nutrient dynamics is controlled by the spatial parameters of lake morphometry, depth and fetch, and bottom substrate. Understanding phytoplankton-nutrient dynamics in Lake Okeechobee requires information about the spatial and temporal differences in the nutrients limiting phytoplankton biomass.

Application of the limiting nutrient concept, to model phytoplankton-nutrient relationships, has focused on nitrogen (N) and phosphorus (P) loading or the in-lake concentrations of N and P (Canfield 1983, Dillon and Rigler 1974, Jones and Bachmann 1976). Empirical models of phytoplankton-nutrient relationships can be confounded by internal nutrient recycling, luxuriant uptake and storage of P by phytoplankton (Fitzgerald and Nelson 1966, Smith and Kalff 1981, Wynne and Berman 1980) or limiting effects of light (Carper and Bachmann 1984, Dokulil et al. 1979, Hoyer and Jones 1983). An experimental approach, the nutrient enrichment bioassay (NEB) can be used to isolate confounding factors and provide an independent method of verifying empirically defined relationships.

In Lake Okeechobee, observation of large-scale algal blooms in the 1980s raised concerns about excess P loading from anthropogenic sources (Kratzer and Brezonik 1984, Lake Okeechobee Technical Advisory Committee 1986). Janus et al. (1990) reported, based on total N (TN):total P (TP) ratios, chlorophyll *a* (Chl-*a*) data (Janus and Vollenweider 1981) and P load models (Vollenweider 1975, Vollenweider and Kerekes 1982), that "Okeechobee has shown a number of symptoms indicative of increasing eutrophication in recent years." They concluded that phytoplankton were P limited and that a reduction in phytoplankton biomass could be attained by a reduction in P loading. Canfield and Hoyer (1989), in an opposing opinion, stated, "Based on the water quality data now available, there is no strong evidence that Lake Okeechobee is in a phase of transition to a higher trophic state or that nutrient control programs can lower the trophic status of the lake." Kratzer and Brezonik (1984) and, later, Schelske (1989) concluded that Lake Okeechobee has complex spatial and temporal dynamics that preclude simple whole-lake modeling efforts.

The controversy about factors responsible for the recurrence of major algal blooms led to a five-year Lake Okeechobee Ecosystem Study (LOES), beginning in 1988. The LOES project included a study of N and P as limiting nutrients to phytoplankton growth, which is presented in this chapter. The NEB method, using natural phytoplankton assemblages, was chosen to assess limiting nutrients.

The objectives were to 1) develop qualitative limiting nutrient classifications from NEB of natural phytoplankton assemblages and 2) identify spatial and temporal distribution of qualitative limiting nutrient classifications.

Methods

Lake Okeechobee, located in southern Florida (27° 00′N,80°50′W), is North America's largest subtropical lake with an area of 1732 km^2 (Fig. 2-1). This polymictic lake's shallow depth (mean depth = 2.7 m) and flocculent sediments lead to substantial abiotic turbidity (Aumen 1994). Primary water inflows are from the north of the lake and outflows are to the south. Total P and TN concentrations (Table 2-1) place the lake in the eutrophic classification based on established guidelines (Carlson 1977, OECD 1982).

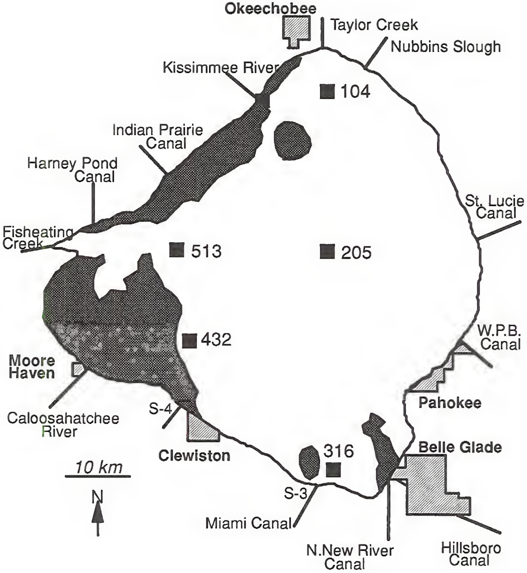


Fig. 2-1. Locations of stations in Lake Okeechobee where monthly samples were collected for nutrient enrichment bioassays. Water inflows and outflows and municipalities are noted on the map. The shaded areas represent littoral zones of extensive vegetation.

Table 2-1. Average values for some morphometric and chemical parameters for sample stations. Data are from monthly integrated water samples collected from January, 1990 through December, 1992 as part of the Lake Okeechobee Ecosystem Study (unpublished data). Standard deviations are reported in the (). The number of samples represented by the mean values are 34, 34, 33, 34 and 31 for stations 104 through 513 respectively.

STATION	MEAN DEPTH (m)	SUBSTRATE TYPE	TP (mg/L)	TN (mg/L)	CHL-*a* (µg/L)	SECCHI DEPTH (m)
104	3.05 (0.73)	mud	0.106 (0.028)	1.720 (0.501)	31.2 (16.0)	0.47 (0.21)
205	3.88 (1.01)	mud	0.101 (0.039)	1.521 (0.501)	22.7 (10.5)	0.32 (0.16)
316	1.48 (0.51)	peat	0.041 (0.029)	1.590 (0.495)	29.5 (24.0)	0.76 (0.47)
432	2.15 (0.62)	sand	0.054 (0.033)	1.555 (0.449)	36.4 (21.0)	0.62 (0.30)
513	2.61 (0.76)	sand	0.060 (0.033)	1.487 (0.462)	37.6 (21.0)	0.63 (0.34)

Sampling Procedures

Nutrient enrichment bioassays were performed monthly from January 1990 through December 1992 on water samples collected from five stations in Lake Okeechobee (Fig. 2-1). These stations represented five spatially distinct pelagic zones: 1) the north lake area (station 104) where inflows from the Kissimmee River, Taylor Creek-Nubbin Slough and Indian Prairie Canal provide the most potential influence by external nutrient sources; 2) the central area (station 205) where mud bottom substrate and wind-driven mixing provide the mechanism for potential nutrient recycling as well as increased possibility of light limitation; 3) the south area (station 316) with hard bottom substrate and some macrophytes where gravity outflows or periodic back-pumping of water from agricultural lands can cause changes in nutrient availability; 4) the southwest area (station 432) at the littoral-open water interface where nutrient flux from the littoral zone may occur; 5) the northwest area (station 513) at the mouth of Fisheating Bay which receives outflows from Fisheating Creek, Harney Pond Canal and extensive littoral areas. Table 2-1 gives a summary of the average morphological conditions and total nutrient concentrations by station.

Integrated water column samples were collected from the surface to 0.1 m from the bottom using 49.2 mm diameter PVC (schedule 40) pipe with stopper (Phlips et al. 1993a).

Several tubes of water were collected to fill a 20 L carboy at each station. Samples were collected before noon and transported the same day to the University of Florida, Gainesville. The carboys were covered with dark plastic bags to shield samples from excess light and transported in an air-conditioned vehicle to reduce temperature fluctuations. Assays were set up in the laboratory the morning after samples were collected.

Experimental Design

The experimental design was a 2x2 factorial with fixed effects as previously described by Aldridge et al. (1993). The independent fixed variables were nutrient type (N and P). Each nutrient was tested at two levels, ambient and enriched. The dependent variable was algal biomass. Statistical significance was accepted at $\alpha = 0.01$ unless otherwise noted.

Assays were done under laboratory conditions in 500-mL Erlenmeyer flasks with 300 mL of water. Treatments (in triplicate) were control (no additions), N addition (400 µgN/L), P addition (40 µgP/L) and a N + P (N+P) addition (400 µgN/L + 40 µgP/L). Nitrogen was added as KNO_3 and P was added as K_2HPO_4. Nutrient additions followed recommendations of Schelske (1984) that enrichments should be "realistic" and no more than twice the maximum ambient concentrations. Incubation was done in temperature-controlled water baths with bottom illumination. Incubation temperature mimicked, as closely as possible, ambient lake temperature on each sampling date, ranging from 20° to 30° C. Light intensity was fixed at 120 $\mu E/m^2/s$. Photoperiod was 12/12 dark/light hours, respectively, from October through March and 11/13 dark/light from April through September. Contents of flasks were thoroughly mixed once a day prior to sampling. A seven to eight mL sample was collected from each flask at the start of the assay and once every 24 h for 96 to 120 h.

Algal biomass was estimated by net *in vivo* fluorescence of Chl-*a* (*IVF*) using a Turner Designs Model 10 fluorometer with a 1-cm path length (Lorenzen 1966). *IVF* was obtained by subtracting the background fluorescence of filtered lake water from the gross fluorescence of each treatment sample. Samples were collected at the same hour each day to minimize circadian cycle effect on *IVF* and were low-light adapted at approximately 20 $\mu E/m^2/s$ for 30 minutes prior to measurement of *IVF*. *IVF* measurements were compared with measurements of extracted chlorophyll *a* (Chl-*a)* (90 percent acetone, APHA 1989) of the initial and final samples for all stations and treatments. Significant increases and decreases in *IVF* were consistently reflected by significant changes in extracted Chl-*a* concentrations.

A nutrient was considered limiting when no growth occurred in the control and addition of that nutrient stimulated algal growth. When algal growth occurred in the control, it was concluded that neither N nor P was limiting and that some component of the phytoplankton community was growing at the time of sampling or some controlled variable, such as light, was more optimal under the assay conditions than in the natural environment.

Preliminary studies showed that *IVF* responses in NEB followed three generalized patterns (Fig. 2-2): (1) the "unlimited" response where there was a significant positive increase in *IVF* for all treatments, (2) single nutrient limitation where a single nutrient treatment and the N+P treatment had significant positive increases in *IVF* while the control and the other nutrient treatment did not increase from the initial measurement and (3) co-limitation by N and P where the *IVF* of the N+P treatment significantly increased but neither control nor N and P treatments increased from the initial measurement. A fourth response type was later identified where no increase or a decline in *IVF* was observed for all treatments from time zero to the end of the assay. This response was termed limitation by some factor other than N, P or the control variables.

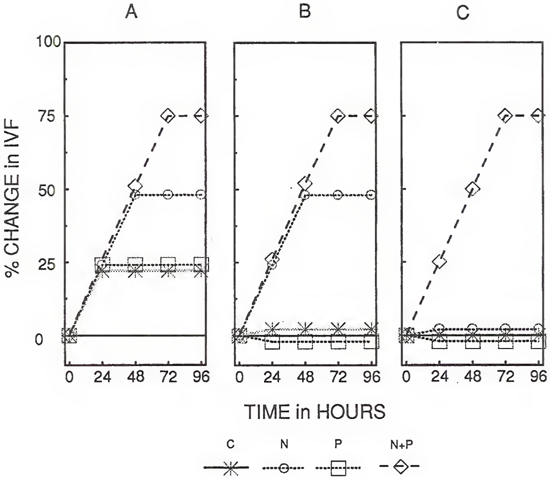


Fig.2-2. Classification examples of three generalized responses to nutrient enrichment bioassays. The classifications are based on the 24-hour response as described in the text. A) not limited by N or P. B) Single nutrient limited. C) Co-limited. C = Control treatment, N = nitrogen addition, P = phosphorus addition, N+P = addition of N and P. Differences of $\leq$ 5% are deliberately made to visualize individual lines.

In 162 of 166 assays, the 24-h response identified the limiting nutrient. Responses at subsequent sampling, 72 and 96 hours, confirmed the limiting nutrient classification at 24 hours. Thus, the 24-hour response was used to assign limiting nutrient classifications. The classification for the 24-hour response was determined using boolean logic as follows:

IF $C_{24} > C_0$ then LC = "U"
IF LC NOT U and $N_{24} > C_{24}$ and $P_{24} \leq C_{24}$ then LC = "N"
IF LC NOT U and $P_{24} > C_{24}$ and $N_{24} \leq C_{24}$ then LC = "P"
IF LC NOT U OR N OR P and $N+P_{24} > C_{24}$ then LC = "N+P"
IF LC NOT U OR N OR P OR N+P then LC = "X"

Where C_x, N_x, P_x, $N+P_x$ were *IVF* responses to treatments at time x: C_0=control at time 0, C_{24}=control at 24 h, N_{24}= N at 24 h, P_{24}= P at 24 h, $N+P_{24}$= N+P at 24 h. LC= limiting nutrient classification where U= growth in the control, N= N limited, P= P limited, N+P= N and P co-limited and X= other limiting factor. A ">" indicates a significantly greater value based on Student's *t*-test results. A 48-hour classification was also made using the same logic.

Treatments were run in triplicate, but there were times, usually at 48 hours, when not all replicates were sampled. Estimated maximum standard deviations for treatments and sample times with n<3 were calculated from analyses of the pooled within treatment variances. Analyses of within treatment variances for treatments where n=3, normalized as coefficient of variation (*C.V.*), showed no significant difference of *C.V.* between stations or treatments for the same sample time. However, there was significant difference in *C.V.* between sample times for all stations and treatments. The mean *C.V.* and 95% confidence interval for the 24-hour and 48-hour samples were 2.23±0.25 (n=356) and 4.46±1.26 (n=86), respectively. Based on these results, an estimated maximum standard deviation ($S_{max-est.}$) for each treatment and sample time where n<3 was calculated as

$$S_{max-est.} = (X * C.V._{95\%C.I})100^{-1}$$

where "X" = the mean of the two replicates or the value from a single sample, $C.V._{95\%C.I.}$ = the upper 95% confidence limit for *C.V.* of the associated sample time as determined from a composite of all samples for that sample time.

Analysis of spatial and temporal homogeneity and independence for the frequency of limiting nutrient classifications, by station or time, respectively, was done using the frequency analysis of SAS STAT Version 6.04 (SAS 1989). Frequency distribution of limiting classificiations were analyzed for intaannual (seasonal) and interannual effects. Seasons were defined as: Winter = December-February, Spring = March-May, Summer = June-August, Fall = September-November.

Results

Relative Frequency of Limiting Nutrient Classification

A total of 166 assays were completed over a three-year period (Table 2-2). Of the 166 assays, 55% were classified N limited, 34% "U," 9% N+P co-limited and 2% limited by some factor "X" other than N and P or any of the control variables. Phosphorus was never a primary limiting factor. Two stations, 104 and 205, were never classified "N+P" or "X."

Comparison of classifications at 24 h and 48 h was used as an indicator of the severity of limitation. A reduction in the frequency of "U" at 48 h, from 34% to 18%, and a concurrent increase in N limited classification, from 55% to 73% (Table 2-2), indicated a growth-driven depletion of biologically available N between 24 h and 48 h. This result also indicated that sufficient biologically available P was present to sustain phytoplankton growth without forcing secondary P limitation.

Two types of anomalies were observed in comparing the 24-hour to 48-hour classifications (Table 2-3). In four assays, N limitation at 24 hours was followed by a 48-hour "U" classification. This occurred twice for station 513 and once each for stations 205 and 316. In six assays, the 24-h classification was "N+P" co-limited and the 48-h classification was N limited. Three of these assays were from 513, two from 316 and one from 432. The low incidence

Table 2-2. Relative frequency for limiting nutrient classification for five stations in Lake Okeechobee determined by nutrient enrichment bioassays. Limitation classified based on the growth response after the first 24 and 48 hours. N= nitrogen, P= phosphorus, NP= N and P co-limitation, U= growth in the control, X= limited by something other than N or P. Percent values rounded to nearest %. The term "WHOLE LAKE" refers to the average for all stations at 24 or 48 hours.

Limiting Nutrient Classification

	U	N	P	NP	X
STATION	% (n)	% (n)	% (n)	% (n)	% (n)
		24 Hours			
104	47 (16)	53 (18)	0	0	0
205	65 (22)	35 (12)	0	0	0
316	18 (6)	49 (16)	0	24 (8)	9 (3)
432	24 (8)	68 (23)	0	8 (3)	0
513	13 (4)	74 (23)	0	13 (4)	0
WHOLE LAKE	34 (56)	55 (92)	0	9 (15)	2 (3)
		48 Hours			
104	17 (5)	80 (24)	0	0	3 (1)
205	37 (11)	63 (19)	0	0	0
316	11 (3)	55 (15)	0	30 (8)	4 (1)
432	10 (3)	86 (25)	0	3 (1)	0
513	11 (3)	81 (22)	0	8 (2)	0
WHOLE LAKE	18 (25)	73 (105)	0	8 (11)	1 (2)

of anomalies was not considered significant when compared to the total number of assays.

Limiting nutrient Classification by Station

The frequencies of limiting nutrient classifications varied among stations, which indicated the responses from one or more stations were significantly different (*Chi-sq* test for homogeneity and independence) than other stations. Table 2-3 presents all 24-hour and 48-hour classifications by station and month.

Nitrogen limitation was found most frequently at the western stations, 432 and 513, 68% and 74%, respectively (Table 2-2). These two stations also had similar, 9-13%, frequencies of "N+P" co-limitation. The similarity between these stations was more pronounced at 48 h when there was an increase in the frequencies of N limitation and a corresponding decrease in the "U" classification. No seasonal pattern was evident for either station for any of the three years.

The frequency of N limitation, after 24 hours, was similar for the northern station, 104, and the southern station, 316, 53% and 49%, respectively (Table 2-2). However, these two stations differed in the frequencies of "U" and "N+P" co-limited classifications. Station 104 had a higher frequency of "U" classification than station 316, 47% and 18%, respectively, while station 316 had a greater

Table 2-3. Limiting nutrient classification by station and time in Lake Okeechobee determined by nutrient enrichment bioassays. Limiting nutrient classification based on the growth response after 24 and 48 hours. N= nitrogen , P= phosphorus, NP= N and P co-limitation, U= growth in the control, X= limited by something other than N or P.

	STATIONS				
DATE (YYMMDD)	104 (24h/48h)	205 (24h/48h)	316 (24h/48h)	432 (24h/48h)	513 (24h/48h)
900111	U U	U U	U N	U N	. .
900215	N N	U U	U U	N N	. .
900315	U U	U U	N N	U U	. .
900411	N .	U .	U .	U .	N .
900510	N N	U U	NP NP	U N	NP N
900606	N N	N U	NP N	U U	U NP
900711	N N	N N	NP NP	N N	NP N
900816	U N	N N	X .	N N	U N
900913	N N	N N	X X	N N	N N
901019	N N	U U	. .	N N	N N
901114	N N	U N	N N	N N	N N
901212	U U	U U	N N	N N	N N
910109	U U	U U	U NP	N N	NP NP
910213	U N	U U	U NP	N N	N N
910313	N N	U N	N N	U N	U N
910410	N N	U N	N N	U U	N N
910508	U .	U .	X .	NP .	N .
910600	. .	. .	. .	. .	. .
910710	N N	N N	NP NP	N N	N N
910821	U N	N N	NP NP	NP N	N N
910911	U N	N N	NP NP	N N	N N
911009	U N	U N	NP N	N N	U N
911113	U N	U U	N N	N N	N N
911206	U N	U N	N U	N NP	N N
920114	U .	U .	N .	N .	N .
920212	U U	U U	N N	U N	N U
920311	U N	U N	N N	N N	N U
920415	N .	U .	N .	NP .	N .
920513	N N	U N	N N	N N	N N
920617	N N	N N	N N	N N	N N
920715	U N	N N	N N	N N	N N
920827	N N	N N	U U	N N	N N
920916	N X	N N	N .	N N	N N
921000	. .	. .	. .	. .	. .
921113	N N	N N	NP NP	N N	NP N
921210	N N	U N	N N	N N	N U

frequency of "N+P" classification than station 104, 24% to 0%, respectively. The differences between these two stations was highlighted by comparing the classification frequencies at 48 hours. The frequency of N limitation increased for station 104 at 48 hours, while station 316 showed an increase in N+P co-limitation at 48 hours. These results indicated a greater ratio of biologically available P to phytoplankton biomass at station 104 compared to station 316. No seasonal effects were discernible for station 104. There was a seasonal incidence of "N+P" and "X" classifications at station 316 during the summer and fall months of 1990 and 1991, but this pattern was not present in 1992.

The center lake station, 205, had the greatest frequency of "U" classification (65%) and the lowest frequency of N limited classification (35%)(Table 2-2). The 48-hour classification showed a reversal in the frequencies of "U" (37%) and N-limited (63%) classifications. This result suggested a rapid, growth induced, depletion of biologically available N such that N became limiting after 24 hours under laboratory conditions. Station 205 was the only station to show a strong seasonal pattern, where N limitation was prevalent during the summer months, June through September, and was classified "U" the remainder of the year. An exception to this pattern was observed in 1992 when the N limitation season extended through November.

Interannual Distribution of Limiting Nutrient Classifications

Analysis of interannual frequency distributions for limiting nutrient classifications revealed significant differences between years. The year 1992 had a greater frequency of N-limited classification (73%) and less frequent "U" classification (22%) than the prior two years (Table 2-4). On a lake-wide basis, 1990 and 1991 had similar frequency of N limited classification (45-48%) and "U" classification (39-40%) at 24 hours. These differences between years were not as apparent in the 48-hour samples, where the frequency of N-limited classifications ranged from 66% to 80% and the frequency of "U" classification ranged from 12% to 26% (Table 2-4).

When differences between years for each station were analyzed, two groups of stations were identified from the 24-hour data (Table 2-3). At stations 316, 432 and 513, there was a progressive increase in the frequency of N limitation for each year from 1990 through 1992 with the greatest increase occurring between 1991 and 1992. In contrast, stations 104 and 205 showed a decline in the frequency of N limitation from 1990 to 1991 and then an increase in N limitation in 1992.

Table 2-4. Interannual distribution for limiting nutrient classifications reported as relative frequency of occurrence. Limitation classified based on the growth response after the first 24 hours and significant growth between 24 and 48 hours. N= nitrogen , P= phosphorus, N+P= N and P co-limitation, U= growth in the control, X= limited by something other than N or P. Percent values rounded to nearest %.

Limiting Nutrient Classification

	U	N	P	N+P	X
YEAR	% (n)	% (n)	% (n)	% (n)	% (n)
			24 Hours		
90	39 (22)	48 (27)	0	9 (5)	6 (2)
91	40 (22)	45 (25)	0	13 (7)	2 (1)
92	22 (12)	73 (40)	0	5 (3)	0
			48 Hours		
90	26 (13)	66 (3)	0	6 (3)	2 (1)
91	12 (7)	74 (37)	0	14 (7)	0
92	14 (6)	82 (36)	0	2 (1)	2 (1)

Discussion

Assessment of NEB

Dodds and Priscu (1990) compared methods for assessment of nutrient deficiency in phytoplankton and found that measurement of a growth response was necessary to make a definitive statement regarding nutrient deficiencies. Although phytoplankton growth was followed for five to six days, growth responses to nutrient enrichments in my experiments were generally manifested within 24 hours. The time required to obtain a measurable growth response is a function of initial biomass and growth. Initial biomass is important because the ability to resolve detectable changes in algal biomass depends on the quantity of biomass. Growth rate is partially dependent on temperature and light availability. In this study, light was a control variable and water temperatures were always greater than 20°C. The potential for high growth rates at high standing crop of phytoplankton (usually > 20 µg/L Chl-*a*) facilitated detection of significant changes in algal biomass. This rapid response minimized some sources of potential error such as container effects and phytoplankton community structural shifts that may be associated with longer incubation in this type of assay (Schelske 1984).

When discussing the utility of NEB, as applied in this study, it is important to put in perspective what factors were not assessed. Neither composition of the phytoplankton community nor the effect of grazing pressure from zooplankton was specifically addressed. The limiting nutrient classification was determined strictly on the basis of quantitative changes in phytoplankton biomass and was thus insensitive to qualitative changes in phytoplankton which, in any case, were minimized by the short response time of 24 hours. Grazing pressure was assumed equal across all treatments, and therefore any significant differences found between treatment responses were considered a function of the experiment variables. Unequal grazing in small sample volumes, such as used in these assays, commonly is due to unequal distribution of large grazers, which would be reflected as high within treatment variance. Because within treatment variance was < 5% for 24-hour and 48-hour samples, it was concluded that the assumption of equal grazing pressure was valid.

The physiological history of the phytoplankton is dynamic and therefore NEB results are directly indicative only for those specific times when the samples were collected (Schelske et al. 1978). Any extrapolation of limiting nutrient classifications to times between samples is subject to the relationship of short-term variance (hours to days), being a function of chaotic interactions of all environmental factors, and significantly detectable long-term (months to years) variance. If short-term variance is greater than long-term variance, then no predictable temporal effects should be discernible. Because interannual and indications of seasonal variation (station 205) in limiting nutrient classifications were found, it can be concluded that there is one or more long-term temporal functions in the nutrient dynamics of Lake Okeechobee.

The same logic can be applied to the analysis of spatial effects on limiting nutrient classification. If a lake is spatially homogeneous, then no spatial organization in the limiting nutrient classification frequencies should be detectable. Because there was a significant difference between one or more stations, some spatial factor or factors affecting limiting nutrient dynamics is indicated.

Spatial and Temporal Patterns

Phlips et al. (1993a, In Press-b) defined four ecological zones in Lake Okeechobee: 2-3 m depth mud bottom, > 3 m depth mud bottom, 2-3 m depth hard bottom without vegetation, and littoral fringe with hard bottom. Their results were based on discriminant function analysis of mean light availability, Chl-*a*, TN and TP concentrations. Each of the five stations I selected for my research represented a specific area of the lake with characteristic morphological features reflected in the ecological zones defined by Phlips et al. (1993a, In Press-b). My results, relating the patterns of limiting factors to lake morphology, concur with the ecological zone concept.

The results of this study indicate that Lake Okeechobee has both spatial and temporal heterogeneity in limiting factors with N limitation being most prevalent. The high frequency of N limitation in Lake Okeechobee is similar to the situation in another shallow eutrophic Florida lake, Lake Apopka (Aldridge et al. 1993). Tropical lakes, in general, seem to be N limited according to a study by Henry et al. (1985). They reported, in a survey of the literature, that 38 of 41 tropical lakes were found to be N or N and P limited. Nitrogen limitation in Lake Okeechobee is corroborated by the high levels of N_2 fixation reported by Phlips and Ihnat (In Press). High rates of N_2 fixation were found in all but the center lake stations. This follows the regional differences I observed in the frequency of N limitation. Havens (1994) reports similar patterns of N limitation in Lake Okeechobee, determined by deviations in trophic state indices.

The predominance of N limitation can be informative about the status of P in the system. Schelske (1984) reported excessive loading of P may induce N limitation by increasing the demand for N until available forms of that nutrient are depleted. Because biologically available P was found in excess to phytoplankton community demands, it is assumed that the P loading from external sources or internal recycling was in excess to the biological demand.

The second most common classification was "U", particularly at the center lake station and, to a lesser extent, the north station. There are two possible interpretations of this "U" classification: 1) algal biomass was increasing at the sample site at the time of sampling and continued to increase in the laboratory, or 2) *in situ* algal growth was limited by factors other than N or P availability. In either case, algal growth occurred in the laboratory control treatments. Chlorophyll data from associated work indicated that the extended periods of "U" classification for the center lake station, 205, were not typically associated with subsequent Chl-*a* increases (Phlips et al. In Press-a,b), pointing toward the latter conclusion. Because the "U" classification is defined as phytoplankton growth in the control treatment, it can be concluded that some controlled variable was improved in the laboratory to stimulate growth. Of the controlled variables, the light in the laboratory was greater than mean light availability in the mixed layer at station 205 and, therefore, was an optimized factor in the laboratory. Station, 205, with the highest frequency of "U" classification, is located in the area of the lake with the highest suspended solids and the lowest light availability in the mixed layer (1993, In Press-b, -c).

The findings of Phlips et al. (In press-b, -c), in conjunction with the responses observed in this study, suggest that the "U" classification is reflective of an initially light limited condition. Because light was not an experimental variable in this study, no direct inference of light limitation can be made. To test whether light is a limiting factor it would be necessary to run a bioassay with light as an independent variable at different levels of intensity while mimicking the light quality found at each station.

Beyond the general observation that N is the most common limiting nutrient in Lake Okeechobee, these results indicate spatial and temporal patterns in limiting nutrient classifications. Nitrogen and N+P co-limitation were most common at the western (432 and 513) and southern (316) stations. The frequency of N limitation at these stations increased in each of the three years of the study, which coincided with significant changes in lake stage. The lake stage at the beginning of 1990 was below normal due to preceding years of drought. The drought continued through the fall, 1990, after which above normal rainfall occurred. This change resulted in an approximate 1.0 m increase in lake stage between June and August 1991, after which average 1992 lake stage remained higher than the preceding two years.

Variation in lake stage was associated with the frequency of "N" classification at stations 432 and 513, where the lowest frequency of "N" coincided with the lowest lake stage in 1990. The year 1991 was a transition year from low to high lake stage and coincidental increase in "N" classification. The year with the highest annual average lake stage and highest frequency of "N" classification (77%) was 1992. Two possible explanations for this observed relationship are that increased hydrologic loading was accompanied by increased P loading thus driving the algae to N limitation or that a change in lake stage altered light availability and bottom-substrate interaction. Stations 316, 432 and 513 shared similar bottom substrate type (sand or peat) which was not readily suspended in the water column by wind energy. The low potential for suspension of non-algal solids in the water column and the low frequency of "U" classification at these three stations suggest the potential for light limitation was low.

It is interesting to note that station 316, at the south end of the lake and farthest from the major external loads, is the station with the highest frequency of N+P co-limitation and the lowest standing crop of phytoplankton. Other studies have indicated a decreasing gradient of P concentrations from north to south in the lake (Schelske 1989, Phlips et al. 1993a, In press-a, -b) which supports the hypothesis that P from external loading and subsequent internal recycling is being sequestered before reaching the south end of the lake. This pattern is further supported by

Reddy et al. (1991), who reported that the mud sediments in the center of Lake Okeechobee are a net sink for P.

The north and central stations, 104 and 205, share common mud-bottom substrate and a high potential for non-algal suspended solids in the water column. Phlips et al. (In press-c) calculated mean light availability (Stefan et al., 1976) for stations 104 and 205 and found both stations to have below critical mean light availability for phytoplankton growth during a majority of the year and therefore a high probability for light limitation. This result coincides with the high frequency of "U" classification found for these two stations.

The seasonal nature of N limitation during the summer months at station 205 coincides with the annual peak in chlorophyll at that station and the months of lowest wind energy (Maciena and Soballe 1990, Phlips et al. In Press-b). The seasonal coincidence of N limitation and low wind energy leads to a scenario where, during all but the summer months, there is sufficient wind energy to suspend non-algal suspended solids from the bottom mud causing light limitation of the phytoplankton community. A decrease in wind energy during the summer months would allow non-algal suspend solids to drop out. Light limitation would be relaxed and N would become the factor limiting phytoplankton standing crop.

Implications of Limiting Nutrient Classifications

The spatial and temporal distribution of limiting nutrient classifications reported in this study and other corroborative data, showing regionality of various trophic state indicators (Phlips et al. 1993a, In Press-b), indicate that different areas of Lake Okeechobee may be differentially sensitive to N and P limitation and, therefore, loading. Load model predictions of lake TP concentration and subsequent prediction of phytoplankton biomass using a TP-Chl-*a* relationship are usually based on the assumption that the lake is a homogeneous mixed reactor (Dillon 1974). Because data presented on spatial heterogeneity show that this assumption is violated in Lake Okeechobee, some modification in modeling strategy must be made to account for the spatial heterogeneity in growth limiting factors.

Six of eight long-term monitoring stations established by the South Florida Water Management District, from which Janus et al. (1990), Canfield and Hoyer (1988) and Kratzer and Brezonik (1984) used data for their model analysis, are located in open water areas similar to station 205 of this study. Using only limiting nutrient classifications from station 205 would lead to the conclusion reached by Canfield and Hoyer (1988), that neither N nor P is predominantly limiting and therefore control of external loads for P might not significantly affect phytoplankton biomass in the lake.

A majority of the eight long-term monitoring stations are relatively insensitive to load changes. Attempts to assess the effectiveness of nutrient load management using data from these stations might not reflect actual effects for the whole lake. As this study indicates, there are areas in the lake (stations 316, 432 and 513 representing the southern and western areas) which are more sensitive to nutrient limitation by N and P and therefore might reflect greater sensitivity to changes in nutrient loading.

My results show that the implicit assumption of either N or P being the universal primary limiting nutrient in predicting phytoplankton standing crop is not valid in Lake Okeechobee. These findings may explain the difficulties in using P load models or whole-lake means of TN and TP to resolve differences between predicted and actual phytoplankton biomass (Kratzer and Brezonik 1984, Janus et al. 1990). Canfield and Hoyer (1988) found no empirical relationship between TN, TP and Chl-*a*. They concluded that light was the factor controlling phytoplankton biomass in the open water areas of the Lake Okeechobee. My results, derived from an experimental approach, confirms that neither N nor P universally limited phytoplankton biomass in the central lake area and indirectly supports the hypothesis of light limitation. These findings also provide experimental confirmation to Schelske's (1989) hypothesis that Lake Okeechobee has complex seasonal and spatial phytoplankton dynamics that make it difficult to use a simple model approach to project possible responses to different lake management strategies. These results emphasize the need to test assumptions implicit in nutrient-phytoplankton relationships before applying conclusions based on those assumptions for management planning.

CHAPTER 3
SPATIAL AND TEMPORAL DISTRIBUTION OF LIMITING NUTRIENT CLASSIFICATIONS IN LAKE OKEECHOBEE DETERMINED BY DISCRIMINANT FUNCTION ANALYSIS

Introduction

Defining the spatial and temporal distribution of limiting factors that control phytoplankton biomass is essential to understanding the ecology of a lake. The phytoplankton communities in large shallow subtropical lakes, such as Lake Okeechobee, do not necessarily respond equally throughout the lake (Chapter 2, Phlips et al. 1993a) nor display seasonal patterns described in temperate lakes (Wetzel 1983).

Nutrient dynamics in Lake Okeechobee are dominated by five major features: 1) high phosphorus (P) input from the northern drainage basin (MacGill et al. 1967, Lake Okeechobee Technical Advisory Committee 1986), 2) the potential for high nitrogen (N) influx resulting from episodic backpumping from the Everglades Agricultural District south of the lake (Brezonik and Frederico 1975), 3) internal recycling of nutrients supplied from resuspension of flocculent sediments (Canfield and Hoyer 1988, Reddy et al. 1989), 4) flushout of nutrients from the extensive littoral zone on the west side of the lake (Dierberg 1992),

5) resuspension of nutrients incorporated in meroplankton from the water-sediment interface into the water column (Carrick et al. 1993).

Studies of phytoplankton ecology in shallow eutrophic lakes have shown that phytoplankton biomass, measured as chlorophyll *a* (Chl-*a*), may not be directly related to total N (TN) or total P (TP) concentrations. Empirical relationships, using in-lake concentrations of TN and TP to predict the concentration of Chl-*a* in Florida lakes (Canfield 1983), explained less than five percent of the variance in Chl-*a* concentrations in Lake Okeechobee (Phlips et al. In Press-a, -c). Phytoplankton were predominantly N limited or initially unlimited under assay conditions (chapter 2). Phlips et al. (In Press-c) used calculations of mean light availability to show a high potential for light limitation in the central area of Lake Okeechobee. Spatial variability in phytoplankton in Lake Okeechobee indicates it does not respond as a homogeneous mixed reactor (chapter 2). Therefore, it would be useful to define areas of similar factors controlling phytoplankton abundance.

This study utilized discriminant function analysis of nutrient enrichment bioassay results, reported in chapter 2, and a data base of physical and chemical data from the Lake Okeechobee Ecosystem Study (LOES unpublished data) to predict limiting nutrient classifications and map their spatial distribution by seasons and years for Lake Okeechobee.

The objectives of this study were to:

1) Define discriminant functions relating physical and chemical data to each limiting nutrient classification determined by NEB.
2) Map the distribution of limiting nutrient classifications, predicted by discriminant functions, by years and seasons.

Methods

Sampling and Assay Procedures

Nutrient enrichment bioassays were run monthly from January 1990 through December 1992 on water samples collected from five stations in the lake as described in Chapter 2. During the first year of this study, monthly assays were also run on samples from three additional stations: station 112 at the outflow of Taylor Creek in the extreme north, 218 in the south central pelagic region and station 318 midway between stations 218 and 316 (Fig. 3-1). Stations 112, 104, 205, 218, 318 and 316 comprised a north-south transect covering different depth and substrate types whereas the remaining two stations, 432 and 513, were selected to look for potential effects from the western littoral area (station 432) and outflow from Fisheating Bay (station 513).

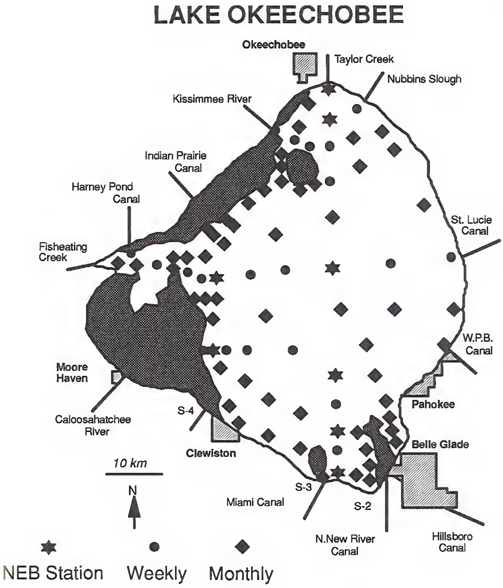


Fig. 3-1. Locations of stations where samples were collected monthly for nutrient enrichment bioassay experiments from Jan. 1990 - Dec. 1992. Locations of stations sampled weekly or monthly during the same years for physical and chemical data are also shown. The shaded areas are highly vegetated littoral zones. Water inflow and out flows as well as municipalities are labeled.

The physical and chemical data set, consisting of 6469 observations from 76 stations collected over three years, was compiled from the Lake Okeechobee Ecosystem Study Project, South Florida Water Management District and the U.S. Meteorological Service. An observation consisted of physical and chemical measurements from a single station on a single date. The variables included Chl-*a*, Secchi disk depth (SECCHI), water temperature, conductivity, TN, TP, total suspended solids (TSS), inorganic suspended solids (ISS), dissolved silica, mean light availability (*Im*)(Stefan et al. 1976), mean monthly wind speed and mean monthly rainfall.

Data Analysis

Data analysis consisted of five steps: 1) identification of significant input variables from the physical and chemical data set using stepwise discriminant function analysis, 2) definition of a discriminant function for each limiting nutrient classification using the classifications from the NEB data set and the input variables identified in step 1, 3) prediction of a limiting nutrient classification for each observation in the physical and chemical data set using the discriminant functions defined in step 2, 4) identification of the dominant classification for each station and 5) mapping the distribution of dominant classifications for the total data set and by year and season. A flow chart for the organization of the analysis is given in Fig. 3-2.

Identification of the appropriate input variables for discriminant function analysis was made using stepwise discriminant function analysis (SAS 1989). Input variables are those variables which, in the context of statistical analysis, define a statistical relationship. This analysis was run on a merged data set compiled from NEB derived limiting nutrient classifications and the corresponding physical and chemical data from the eight NEB stations. Natural log transformations of all variables were also included in the step-wise analysis.

Discriminant function analysis (SAS 1989) was used to define the discriminant function for each limiting nutrient classification. Validity of the discriminant functions was tested by using resubstitution and cross-validation procedures (SAS 1989). The *Chi-Sq* test was used to test for homogeneity of within covariance matrices. Once the discriminant functions were defined, discriminant function analysis was applied to observations from all stations and dates from January 1990 through December 1992 to predict a limiting nutrient classification for each observation. Estimates of the posterior probabilities for an observation being assigned to each of the four limiting nutrient classes were calculated and used to locate and evaluate the sources

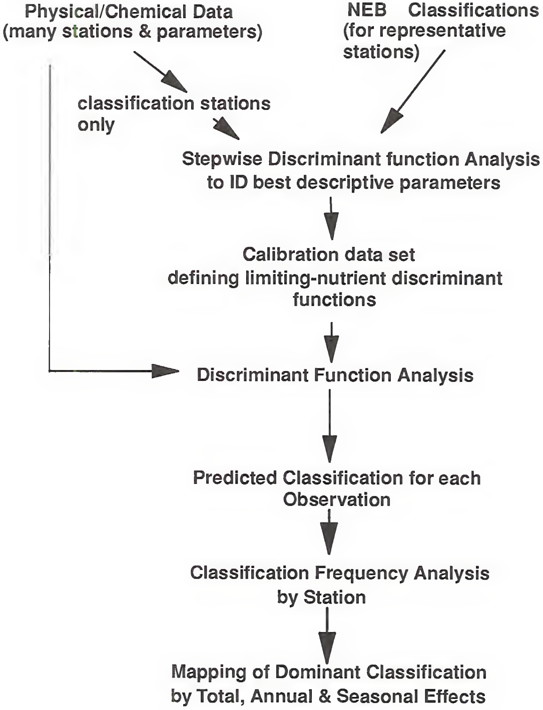

Physical/Chemical Data
(many stations & parameters)
NEB Classifications
(for representative
stations)
classification stations
only
Stepwise Discriminant function Analysis
to ID best descriptive parameters
Calibration data set
defining limiting-nutrient discriminant
functions
Discriminant Function Analysis
Predicted Classification for each
Observation
Classification Frequency Analysis
by Station
Mapping of Dominant Classification
by Total, Annual & Seasonal Effects

Fig. 3-2. Flow chart of the analytical process used to derive the distribution of limiting factors in Lake Okeechobee Florida. See text for explanation.

of error found in the resubstitution and cross-validation analysis.

Because each station had more than one observation, the assigned limiting nutrient classification was the classification with the greatest percent frequency of occurrence. The dominant limiting nutrient designations were plotted on a map of the lake, from which the spatial boundaries of like classified areas were charted by interpolation between points. Where stations had no single clearly dominant classification, the ranking of classification frequencies was used to weigh the interpolation of the boundaries.

The distribution of limiting nutrient classifications was mapped as a composite of all observations and also by seasons and years. Seasons were defined as: Winter = December-February, Spring = March-May, Summer = June-August, Fall = September-November. These seasonal definitions are based on changes from extreme conditions of warm (>25° C water temperature) high rainfall summers in June through August and cooler (<25° C water temperature) high wind winter months of December through February. The months between these extremes are the transitional months defining spring and fall.

Results

Discriminant Function Analysis

The results from 202 nutrient enrichment bioassays, completed over a three-year period, were used to identify discriminant functions for specific limiting nutrient classifications. Discriminant functions were defined for four limiting nutrient class types: "U"= unlimited (ie. sufficient N and P to sustain growth), "N"= N limited, "NP"= N and P co-limited, "X"= limited by something other than N or P. Because there was no occurrence of "P" limitation by itself in the calibration data set, it was not possible to define this classification.

Step-wise discriminant function analysis revealed only four of the 20 input variables (Chl-*a*, SECCHI, *Im* and ISS) contributed significantly to the discriminant functions that defined the limiting nutrient classification. Discriminant function analysis was run with various combinations of Chl-*a*, SECCHI, *Im* and ISS. SECCHI and *Im* were found have similar contributions to the discriminant functions. Because there were more observations with SECCHI than *Im*, SECCHI was selected for inclusion in the subsequent analysis. The contribution of ISS to the analysis was small, partial r^2=0.051, and thus was also dropped. Only Chl-*a* and SECCHI were necessary to form significant discriminant functions which best described the limiting nutrient classifications. The test for homogeneity of within covariance matrices showed significant *Chi-Sq* difference (P<.0001) so within covariance matrices were assumed unequal and quadratic discriminant functions were specified. Quadratic discriminant function analysis was run on the calibration data set using Chl-*a* and SECCHI as the random input variables and limiting nutrient class as the classification variable.

An X-Y plot of SECCHI x Chl-*a* revealed a positively-skewed unimodal distribution with a maximum Chl-*a* value of 155 µg/L and a complementary SECCHI value of 0.5 m (Fig. 3-3). The four areas defined by the discriminant functions of the four limiting nutrient classifications were overlaid on the SECCHI x Chl-*a* plot (Fig. 3-4). The region of low SECCHI, <0.7 m, and low Chl-*a*, < 40 µg/L defined the "U" classification. The region of highest SECCHI, > 1.4 m, and lowest Chl-*a*, < 30 µg/L, defined the "NP" co-limited classification. The "X" classification was associated with the region of the highest ratio of Chl-*a*:SECCHI, and roughly defined as above the line; Chl-*a* = 115 - SECCHI*80. The central region on the SECCHI x Chl-*a* plot, bound by the "U", "NP" and "X" regions, defined the N limited classification.

The probability of assigning an incorrect limiting classification to an observation based on the discriminant functions of SECCHI and Chl-*a* was approximated by the error rates of resubstitution and cross-validation analyses. These error rates were 28.7% and 29.7% for the

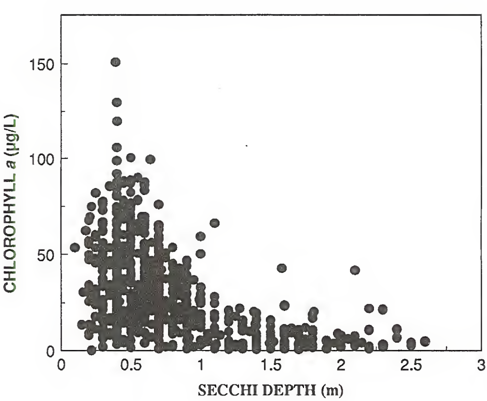


Fig. 3-3. Plot of monthly Secchi depth X Chlorophyll *a* concentration for all observations for three years (1990-92) from the eight nutrient enrichment bioassay stations in Lake Okeechobee, Florida.

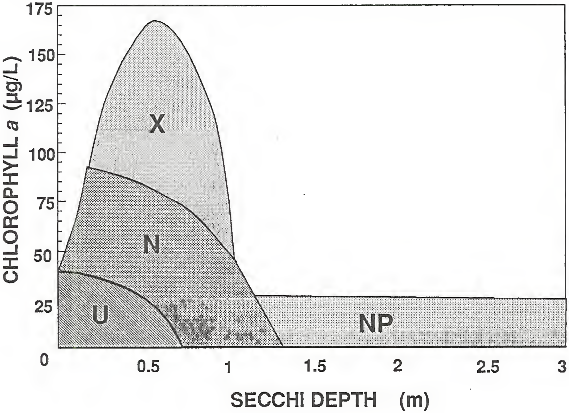


Fig. 3-4. Plot of the discriminant function regions defining the limiting nutrient classifications overlaid on a plot of Secchi depth X chlorophyll *a* concentration. Discriminant functions were derived from nutrient enrichment bioassay experiments and physical and chemical data collected monthly for three years (1990-92) from eight stations in Lake Okeechobee.

resubstitution and cross-validation respectively. To determine if there was any organization to this error, the posterior probability associated with the assigned nutrient classification of each observation was noted on a plot of SECCHI x Chl-*a* (Fig. 3-5). Distinct patterns in the distribution of the posterior probabilities were noted. The lowest probabilities, 0.4-0.5, were associated with those regions on the plot where the limiting nutrient classification boundaries were defined. The observations with the highest posterior probabilities values, 0.8-0.9, were found farthest from the boundary regions. An analysis of the source of error revealed that approximately 90% of those observations in the calibration data set that were misclassified in the cross-validation had a maximum posterior probability of < 0.6 and were located near the boundary areas defining the separation between limiting nutrient classification on the SECCHI x Chl-*a* plot (Fig. 3-4). Thus a rough 90% confidence interval about the boundaries between classifications could be defined as an area equal to ±5 µg/L Chl-*a* and ±0.1 m SECCHI where, outside that boundary area, observations could be expected to be correctly classified with greater than or equal to 90% accuracy. The correct classification for those observations within the classification boundary regions that were misclassified was found to be the classification with the second highest posterior probability. This second most

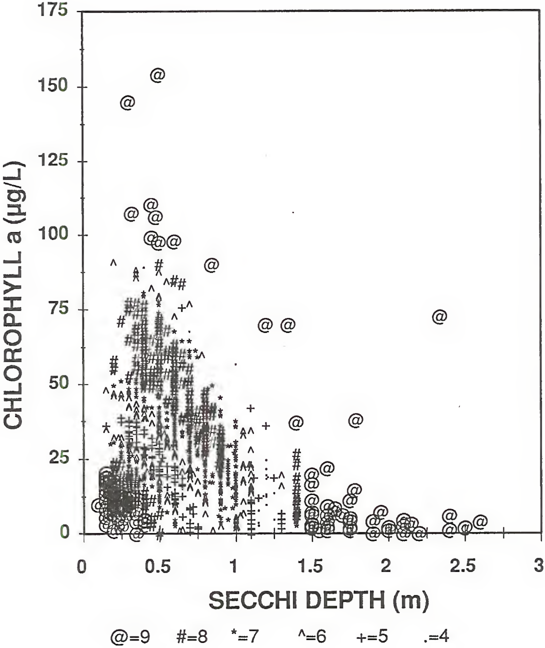


Fig. 3-5. Posterior probabilities overlaid on a plot of Secchi depth X chlorophyll *a* concentration. Posterior probability is the probability of an observation being assigned its dominant limiting nutrient classification by discriminant function analysis. The probability values were rounded to the nearest 10%. The values in the legend are multiples of 10.

probable classification was, in all but a few cases, the classification on the other side of the boundary.

Spatial Distribution of Limiting Classifications

A spatial distribution of predicted limiting nutrient classifications was derived from analysis of physical and chemical data collected over three years (Fig. 3-6). Although four limiting nutrient classifications (U, N, NP and X) were defined, the "X" classification was not plotted because it was never dominant at any station. The "U" and "N" classifications defined two contiguous zones; the "U" zone in the deeper water central and eastern areas of the lake and the "N" zone defining all other open-water areas. No "NP" zone was designated in the three-year data set but "NP" zones were defined in subsequent seasonal and annual analysis.

Patterns among years were not greatly different from the three-year composite (Fig. 3-6). Zones of "NP" appeared in years 1991 and 1992 but were limited to the shallow waters of the extreme South Bay.

Strong seasonal influences in the areal distributions of the classifications were found in the seasonal maps (Fig. 3-7). Spring and winter seasons were similar to the annual distribution maps with the central and eastern shore areas defined by "U" classification and western and southern areas defined as "N" areas. The summer season was characterized

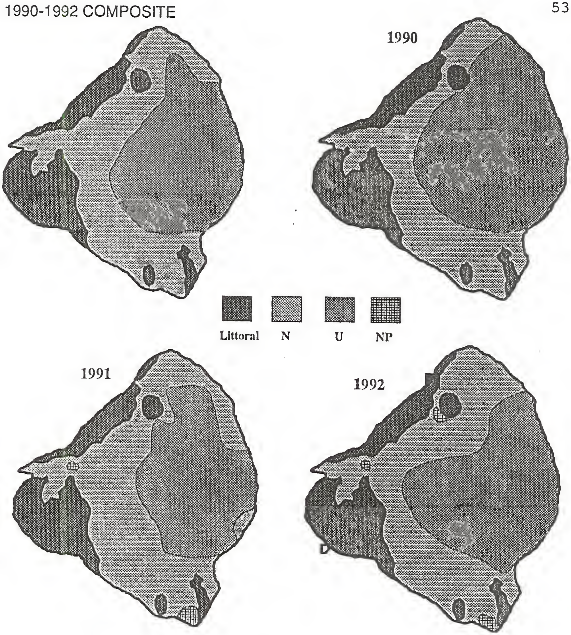


Fig. 3-6. Maps depict the distribution of zones with the same limiting nutrient classifications in Lake Okeechobee for the three-year composite data set and for each year. The zones were defined from the dominant classification for each of 76 stations.

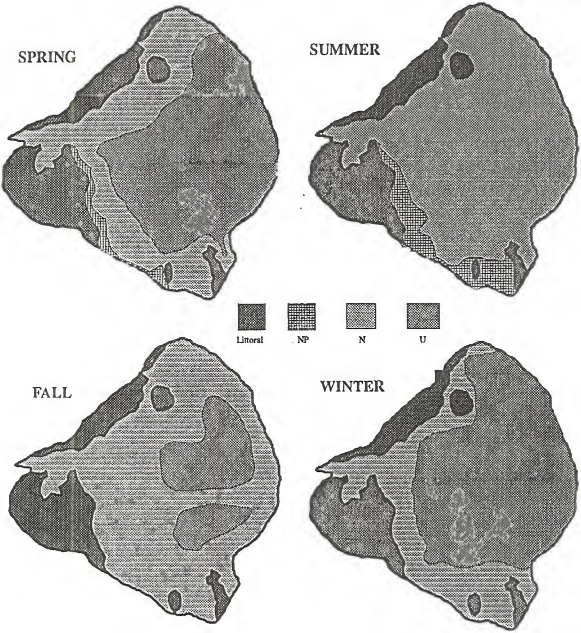


Fig. 3-7. Maps depict the areal distribution of zones with the same limiting nutrient classification by seasons. Zonal areas were defined by the dominant classification predicted by discriminant function analysis for each of 76 stations.

by a complete absence of the "U" classification which was replaced by "N" and an expansion of "NP" along the entire southeastern and southern shores. The "U" zone was found in two areas in the fall. This represented a transition in distribution between the summer and winter seasons.

Discussion

Discriminant Function Analysis and Prediction of Limiting Factors

Discriminant functions that describe specific limiting factors are dependent on the type, quality and quantity of data used to define those functions and are possibly unique to the water body for which they are defined. It may seem counter-intuitive to describe a limiting nutrient type without including nutrient concentrations as input variables. Upon closer inspection of the data, the logic becomes clearer.

Phosphorus was never singularly limiting and thus did not significantly contribute to the definitions of the limiting classifications. This does not imply that P was not a critical nutrient but rather that, during the period of this study, P was in surplus supply relative to the requirements of the planktonic community except in the zones of "NP" co-limitation. If the phytoplankton community were to become limited by the supply of P, it is logical to assume that some form of P should contribute significantly as an input variable in defining discriminant functions for limiting factors. In those cases where P was co-limiting the TP measurement did not reflect that condition.

Nitrogen was the most common limiting nutrient response but TN concentration was not a significant contribution in defining N limited conditions. Lake Okeechobee has high TN, a large percentage is in the form of dissolved organic N, for which no strong relationship to Chl-*a* has been found (Phlips et al. In Press-b). Also, there are large areas of Lake Okeechobee where resuspended tripton contributes a significant percentage of the TN (Canfield and Hoyer 1988). These two large sources of N could obscure the effects of the presence or absence of bio-available forms of N. Some fraction of TN, such as total inorganic N, may have contributed significantly in defining the limiting nutrient functions in this dissertation if data had been available. The results of this analysis indicate that in Lake Okeechobee TN and TP did not define the factors controlling phytoplankton standing crop.

The error associated with the predicted response of the discriminant function analysis was evaluated. The question of accuracy in predicting a limiting-nutrient classification was addressed by the cross-validation analysis. A misclassification error rate of approximately 30% was found. Further analysis revealed an organized pattern in the distribution of misclassification error. Specific areas of

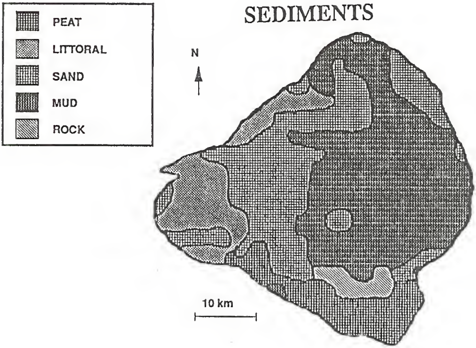
SECCHI-Chl-*a* interaction were identified where higher ($\geq$90%) and lower (<90%) accuracy could be expected and also provided insight to the probable alternate classification for a given observation.

Taking the predicted limiting nutrient classifications for individual observations and using them to predict spatial patterns of distribution automatically sacrifices accuracy. The predicted classification for a given station in the lake is defined as the dominant classification for that station for the specified time factor (seasonal or annual). The error associated with any classification predicted for a station is a function of classification error for all observations from that station. Also, there is a loss of resolution by predicting specific classification for a station where other limitations occur less than a majority of the time.

Boundaries differentiating zones with different limiting nutrient classifications were interpolated between qualitative classifications so it was not possible to assess a statistical confidence interval. The area of closest proximity to a station can be assumed to have the greatest probability of having similar limiting nutrient status as that station and that confidence in the accuracy of the limiting nutrient classification would decrease with increased distance from that station. This would imply that the regions between stations of different classifications would have the greatest error associated with the limiting nutrient assignment. Understanding the sources of error in defining boundaries between areas of different limiting classifications is essential in understanding the utility of such maps. The boundaries are not fixed demarcations of distinct limiting classifications but rather regions of transition between areas of different classifications.

Spatial and Temporal Variation in Limiting Factors

Distributions of limiting nutrient classifications for the three-year composite data set show two contiguous regions of homogeneous classifications: N limited along the north, south and western shores and "U" in the center-lake and eastern shore areas. The "U" area closely coincides with the mud zone delineated by Reddy et al. (1991) and the three meter depth contour (Fig. 3-8). This area is also the zone of highest concentration of non-algal suspended solids (1993a, In Press-b). In an analysis of the relationship between light availability, Chl-*a* and tripton, Phlips et al. (In Press-c) concluded this area of the lake is predominantly light limited. Thus, it was concluded that the "U" classification defined in Chapter 2 is a result of light limitation of phytoplankton production and the distribution of that classification is function of water column depth and bottom substrate type. The distribution of N limited area encompasses open water areas of the lake with


SEDIMENTS

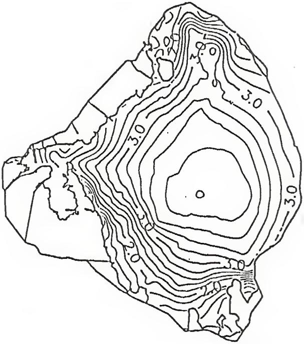

DEPTH CONTOURS

Fig. 3-8. Map of the sediment distribution (from Reddy et al. 1991) and bathymetric map for Lake Okeechobee.

less than three meters water column depth where mean light availability is sufficient to eliminate light limitation.

Analysis of the distribution of limiting nutrient classifications by year showed similar patterns to the three-year composite map with areal extent of the "U" and N limited areas changing but not the general distribution (Fig. 3-6). Because the intraannual variance of the two input variables (SECCHI and Chl-*a*) has been shown to be greater than the interannual variance (Phlips et al. 1993a, In Press-b), differences in areal distribution of "U" and N limited areas are possibly an artifact of the intraannual variance rather than a significant change in limiting classification boundaries.

The strong seasonal pattern of limiting classifications is in sharp contrast with the consistency in distribution patterns between years. The complete disappearance of the "U" classification in the summer months can be attributed to the relaxation of light limitation reported by Phlips et al. (In Press-c). The combination of longer photoperiod, higher light intensity and annual minima for average wind velocity associated with the summer contribute to greater mean light availability in the water column. Increased light availability reduces the potential for light limitation and allows the phytoplankton standing crop to increase until N becomes a secondary limiting factor.

Another seasonal attribute is the development of "NP" co-limited zones along the south and southwest littoral fringe during the spring and summer. These areas of "NP" co-limitation directly coincide with growth of extensive submerged macrophyte vegetation that begins in the spring and peaks in the late summer (Phlips et al. 1993b). Phlips et al. (In Press-a, -b) concluded that the annual Chl-*a* minima observed at stations in these areas during the summer were a result of nutrient competition between phytoplankton and the macrophyte and epiphytic communities. The disappearance of "NP" areas during the fall and winter also coincide with the degradation and eventual disappearance of the macrophyte communities in these areas (Phlips et al. 1993b).

Results of discriminant function analysis presented in this chapter demonstrated that limiting nutrient classifications have spatial and temporal organization. Distributions based either on analysis of the composite data sets or the three annual data sets were similar with little differences among the four distribution maps. By contrast, analysis of data by season showed pronounced seasonal changes in the distribution of limiting nutrient classifications. These results demonstrate the utility of discriminant function analysis. This was advantageous in this study because the limiting nutrient condition for 6469 observations at 76 stations in the lake was predicted from classifications derived from 202 NEBs conducted on samples from eight stations.

CHAPTER 4
USING NUTRIENT ENRICHMENT BIOASSAYS TO DETERMINE PHYTOPLANKTON BIOMASS SUPPORTABLE BY SURPLUS PHOSPHORUS

Introduction

Most efforts at reduction of anthropogenic loading of nutrients into lakes focus on reducing phosphorus (P). There are many studies which have looked at the empirical relationship between phytoplankton biomass, represented as chlorophyll *a* (Chl-*a*), and total P (TP) (Dillon and Rigler 1974, Lund and Reynolds 1982, Oglesby and Schaffner 1975, Sakamoto 1966). These relationships are consistent with the conclusion that P is generally the limiting nutrient in freshwater systems (Schindler 1977). The direct relationship of mean Chl-*a* to mean TP concentrations, plotted from a multi-lake data set (Fig. 4-1A), supports that relationship. In this data set, variance increases with concentration, requiring a log transformation of the data. Other studies done on tropical and Florida lakes (Henry et al. 1985, Canfield 1983) have indicated that P is not necessarily the primary limiting nutrient. Data collected at five stations in Lake Okeechobee from January, 1990 through December, 1992 (Fig. 4-1B) shows no relationship between TP and Chl-*a*.

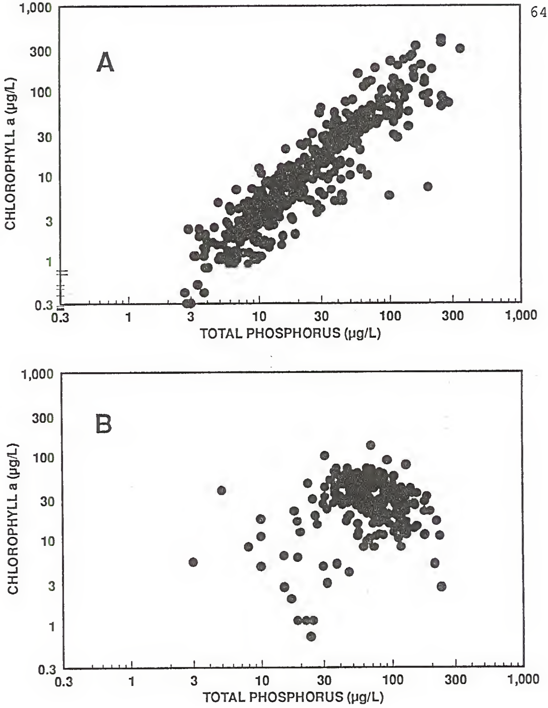


Fig. 4-1. Plot of total phosphorus (TP) X chlorophyll *a* (Chl-*a*). Plot A is for mean TP and Chl-*a* data from 423 lakes from Florida Lakewatch monitoring program and Jones and Bachmann (1976). Plot B is TP and Chl-*a* data for 162 samples from Lake Okeechobee collected at five stations monthly from 1990-92.

Because P was not the primary limiting nutrient for phytoplankton growth in Lake Okeechobee (chapter 2), it is implicit that it was in surplus relative to demand from phytoplankton. Surplus-P is defined here to be all forms of P, either in the water or stored in phytoplankton, that are readily available to phytoplankton for growth but have not been utilized for cell growth at the time of sampling. If no other factors limit phytoplankton growth then phytoplankton biomass would increase until all surplus-P was utilized. This would result in secondary P limitation. It would be of value to know how much surplus-P exists in a system in order to evaluate the scope and feasibility of any plan which attempts to reduce phytoplankton biomass by forcing P limitation.

The two primary forms of surplus-P in the pelagic environment are soluble molybdate reactive phosphorus (SRP) and polyphosphates. The SRP form is equated with the ions $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, which are the most available forms for phytoplankton utilization. It has been shown that phytoplankton can assimilate SRP in excess of immediate growth demands. This surplus-P is stored as inorganic polyphosphates in polyphosphate bodies which can be utilized later for phytoplankton growth (Rhee 1972). Fitzgerald and Nelson (1966) described a hot-water extraction process to hydrolyze and measure these polyphosphates. Although there has been debate as to the exact nature of hot-water extractable phosphates (HEP) (Wynne and Berman 1980), it has been shown to be a good indicator of intracellular stored phosphate (Newman et al. 1994, Pettersson 1980). Surplus-P is equated to be the sum of SRP + HEP.

Evaluation of SRP + HEP, as a direct measurement of surplus-P, requires an independent method of estimation. One method is to use phytoplankton growth response as an indicator of surplus-P in a system. In aquatic systems, where P is the primary limiting factor, there should be no measurable surplus-P and an addition of P should stimulate phytoplankton growth. In systems where surplus-P exists, relaxing those factors limiting phytoplankton growth should stimulate growth until the surplus-P is depleted and P becomes limiting. The increase in phytoplankton biomass from the initial surplus-P environment to a P limited environment should be directly related to the surplus-P in the system (Fig. 4-2).

In this study, nutrient enrichment bioassays (NEB) were conducted to determine the amount of Chl-*a* that could be supported by surplus-P in the phytoplankton community. Chemical measurements were used as an independent measure of surplus-P. The objectives of this study were:

1) Use NEB to estimate the quantity of surplus-P that can support phytoplankton growth.
2) Relate supported Chl-*a*, estimated from NEB, to surplus-P measured chemically.

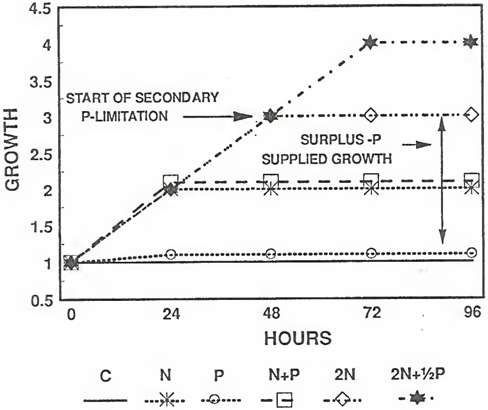


Fig. 4-2. Hypothetical plot of results for a nutrient enrichment bioassay on a phytoplankton assemblage not limited initally by phosphorus (P) availability. C = Control treatment, N = nitrogen (N) addition, P = P addition, N+P = addition of N and P, 2N = double addition of N, 2N+½P = double addition of N and ½ addition of P.

3) Analyze surplus-P data for temporal and spatial trends.

Methods

Nutrient enrichment bioassays were run monthly for three years on integrated water samples collected from five stations in Lake Okeechobee Florida (Figure 2-1). The NEBs were run under laboratory controlled conditions as described in Chapter 2. Nutrient enrichment treatments (in triplicate) were: control (no additions), nitrogen (N) addition (400 µgN/L), phosphorus (P) addition (40 µgP/L) and a nitrogen + phosphorus (N+P) addition (400 µgN/L + 40 µgP/L). Nitrogen was added as KNO_3 and phosphorus was added as K_2HPO_4.

During the course of this study there were times when the N treatment displayed equal growth to the N+P treatment. In these cases it was suspected that algal growth might have been relimited by N rather than forced to secondary P limitation. In order to confirm that secondary P limitation had been obtained, the NEB protocol was modified during the final 15 months of the project to include an 800 µgN/L treatment (twice the original N addition). If response to the 800 µgN/L treatment was significantly greater than the response in the 400 µgN/L treatment then it was assumed that algal growth in the latter treatment utilized all the N addition and became relimited by N.

Phytoplankton growth response was measured as *in vivo* fluorescence (*IVF*) normalized to initial extracted Chl-*a*. For those assays where secondary P limitation was confirmed, the amount of algal biomass increase from the initial biomass to the point of secondary P limitation was defined as the surplus-P supported Chl-*a* (supported Chl-*a*) (Figure 4-2). In those assays where secondary P limitation was not confirmed, the maximum phytoplankton biomass attained in the N or 2N addition treatment was considered to be the closest indicator to actual supported Chl-*a*. The maximum Chl-*a* concentration (total Chl-*a*) that could be potentially supported at the time of sampling was defined as the initial Chl-*a* concentration plus the supported Chl-*a* concentration.

In the final year of the study, a direct chemical estimate of surplus-P was made on the initial samples. SRP was determined on filtered samples using the ascorbic acid-molybdate blue method (APHA 1989). Polyphosphates were determined using a hot-water extraction method reported by Chrost and Overbeck (1987) as adapted from Wynne and Berman (1980). A known volume of sample was vacuum filtered, $\leq$ 300 mm Hg vacuum, through prewashed 0.45 µm polycarbonate filter (Gelman Supor). Each filter with particulate matter was then placed into a screw-top test-tube with 10 ml de-ionized water, 0.055 µS purity, and autoclaved for 60 minutes at 115°C. Autoclaved samples were allowed to cool and then centrifuged at 2000xG for 10 minutes. The supernatant was analyzed for SRP as previously described. The HEP concentration was then normalized to original filtered sample volume. Surplus-P was the sum of the measured SRP and HEP.

Least squares regression analysis was used to compare the NEB derived estimate of supported Chl-*a* with the direct measurement of surplus-P. Surplus-P was defined as the independent variable and supported Chl-*a* as the dependent variable.

Results

In total, 162 assays were completed for which supported Chl-*a* was estimated. During the final year, 59 assays were completed which included a double addition of N (800 µgN/L) to test for N relimitation. In 76% of the latter assays, N was found to be relimiting, after 120 hours incubation, in the single N addition treatment (400 µgN/L).

A distinct spatial grouping of stations was found for the responses of the phytoplankton communities to the two levels of N. In 92% of the assays the three station in the north and center of the lake (104, 205, 513) required the double N addition to force secondary P limitation. The two stations located in the south and southwest (316 and 432) became secondarily P-limited after a single addition of N in 50% of the assays. These results indicate that the supported Chl-*a* data for the first two years (1990-91) have a high probability of being underestimates of the supported Chl-*a* and that supported Chl-*a* data for stations 104, 205 and 513 are almost twice as likely to be underestimated than supported Chl-*a* for stations 316 and 432.

The regression analysis of supported Chl-*a* X surplus-P for the 1992 data revealed a significant linear relationship ($P<.0001$) with a r^2 of 0.52 (Fig. 4-3) where:

supported Chl-a = 17.7 + 0.909*surplus-P

The 1992 surplus-P and supported Chl-*a* were plotted by month for each station (Figs. 4-4, 4-5 and 4-6). Generally, supported Chl-*a* was greater than surplus-P. The seven exceptions, where surplus-P was greater than supported Chl-*a*, showed no apparent pattern due either to station or month. Surplus-P showed a general pattern of maxima in January or February and declining to minima in May. Station 104 had the greatest decline from January to May followed by stations 205, 432 and 513. Station 316 showed the least decline in surplus-P. There was, with the exception of station 205, a general increase in surplus-P after May. No similar pattern was observed for supported Chl-*a*. Neither was there any apparent pattern of covariance between surplus-P and supported Chl-*a* by station nor month.

The averages for TP, surplus-P, supported Chl-*a* and total Chl-*a* are presented for all data and by station in Table 4-1. The average total Chl-*a* for all data indicated

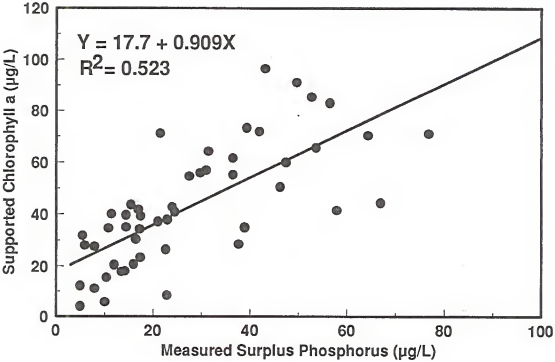


Fig. 4-3. Regression of measured surplus phosphorus and assay derived supported chlorophyll *a*.

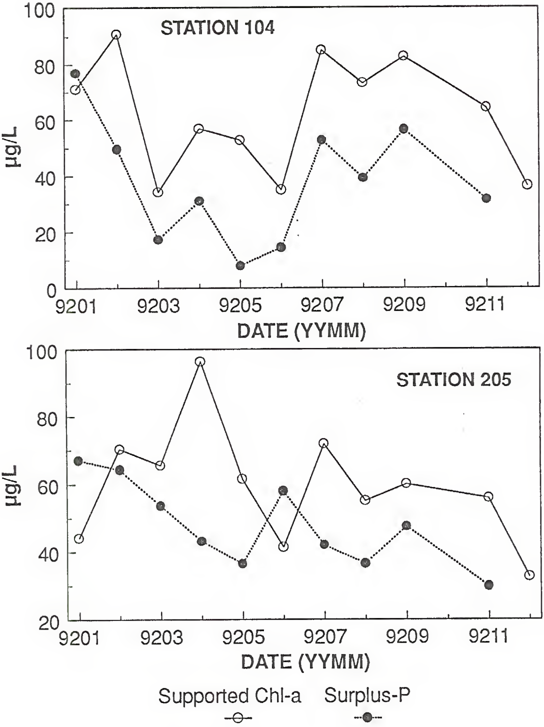


Fig. 4-4. Plot of assay derived supported chlorophyll *a* and measured surplus phosphorus by month for 1992 data from stations 104 and 205 in Lake Okeechobee.

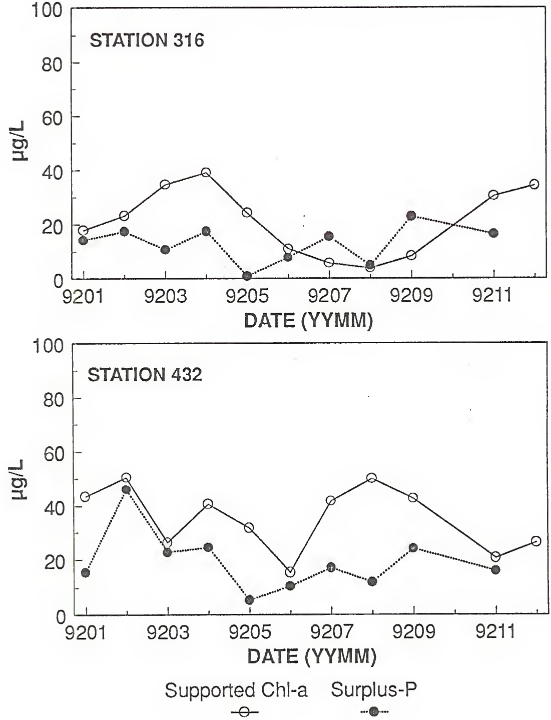


Fig. 4-5. Plot of assay derived supported chlorophyll *a* and measured surplus phosphorus by month for 1992 data from stations 316 and 432 in Lake Okeechobee.

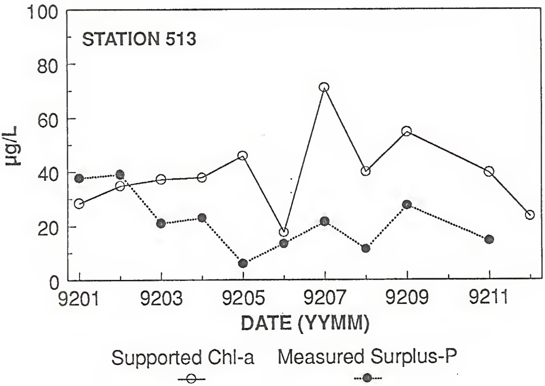


Fig. 4-6. Plot of assay derived supported chlorophyll *a* and measured surplus phosphorus by month for 1992 data from station 513 in Lake Okeechobee.

Table 4-1. Summary of chemically and experimentally derived data for five stations in Lake Okeechobee, FL. Results of chemical measurement of surplus phosphorus (Surplus-P) and total phosphorus (TP) are mean values of monthly samples from July, 1991 through November, 1992. Supported chlorophyll *a* (Chl-*a*) is derived from nutrient enrichment bioassays and total Chl-*a* is the sum of initial Chl-*a* + supported Chl-*a*. Both Chl-*a* terms are reported as mean values for monthly data collected from January 1990 through December, 1992. Standard deviation and sample number (n) are given in the brackets. Coefficient of variation (*CV*) is rounded to the nearest percent. Totals are summary statistics of data from all stations.

STATION	Supported Chl-*a* (µg/L)	Total Chl-*a* (µg/L)	Surplus-P (SRP+HEP) (µg/L)	TP (µg/L)
104	50 CV=46% (23,n=34)	78 CV=35% (27,n=37)	46 CV=48% (22,n=14)	89 CV=27% (28,n=14)
205	48 CV=40% (19,n=31)	71 CV=37% (26,n=31)	55 CV=42% (23,n=15)	100 CV=32% (32,n=15)
316	13 CV=100% (13,n=32)	40 CV=88% (35,n=32)	16 CV=69% (11,n=15)	38 CV=74% (29,n=15)
432	24 CV=83% (20,n=34)	60 CV=58% (35,n=34)	20 CV=45% (9,n=15)	52 CV=50% (26,n=15)
513	26 CV=69% (18,n=31)	68 CV=53% (36,n=31)	23 CV=39% (9,n=15)	60 CV=31% (18,n=15)
TOTALS	32 CV=79% (24,n=162)	64 CV=53% (34,n=162)	31 CV=74% (23,n=74)	67 CV=52% (35,n=74)

that all forms of P utilized or utilizable by phytoplankton in Lake Okeechobee could support an average of 64 µg/L total Chl-*a* with a coefficient of variation (*C.V.*) of 53%. Average supported Chl-*a* comprised approximately 50% of the average total Chl-*a*. For those observations where surplus-P was measured, the TP averaged 67 µgP/L (*C.V.* 52%) and the surplus-P averaged 31 µgP/L (*C.V.* 74%) (Table 4-1). The surplus-P comprised more than 50% of the TP. There was agreement between the lake average surplus-P and average supported Chl-*a* estimates of 31 and 32 µg/L respectively. This overview indicates that, as an average for all data, approximately 50% of the P available to support phytoplankton biomass was not utilized by phytoplankton and this surplus-P comprised approximately half of the TP in the system.

When the results are broken down by station there was an apparent north to south trend with the highest estimates for three of the four parameters (surplus-P, supported Chl-*a*, total Chl-*a*) found at the northern most station, 104, and decreasing to the lowest estimates at the southern most station, 316 (Table 4-1). An exception to this trend was the average TP concentration at station 205 which was greater than that of station 104. Comparing the variances of station averages shows the inverse trend with the greatest *C.V.* associated with station 316 and the lowest with station 104 (Table 4-1).

To determine whether seasonal patterns were present, supported Chl-*a* and total Chl-*a* were plotted over time for the 3-year data set (Figs. 4-7, 4-8, 4-9). Supported Chl-*a* and total Chl-*a* covaried directly with time, indicating Chl-*a* standing crop and surplus-P had a common controlling function. No organized periodicity, for either variable, was observed for stations 104, 205 and 513. Of particular interest was an apparent increase in the average supported Chl-*a* and total Chl-*a* after the spring of 1991 which coincided with a one meter increase in lake stage during the period. The temporal pattern of station 513 was similar to station 205 but with lower amplitude. Stations 316 and 432 showed some seasonal periodicity with minima in the summer months and maxima in the winter months.

Discussion

A number of direct measurements or assay methods can be used to quantitatively estimate the amount of surplus-P in an aquatic system, but many have limitations. Use of assay techniques such as short-term uptake kinetics and alkaline phosphatase activity can be confounded by the presence of heterotrophic organisms or adverse physical/chemical conditions (Dodds and Priscu 1990). The

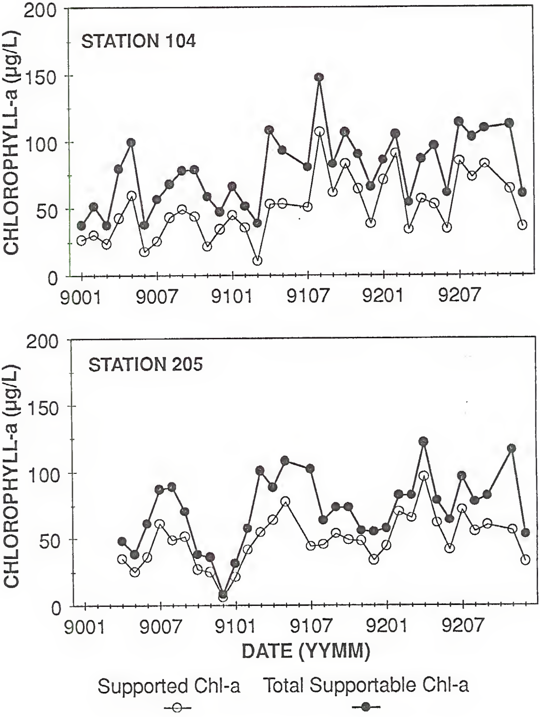


Fig. 4-7. Time series of assay derived estimates of supported chlorophyll *a* (Chl-*a*) and total Chl-*a* (initial Chl-*a* + supported Chl-*a*) for stations 104 and 205 in Lake Okeechobee.

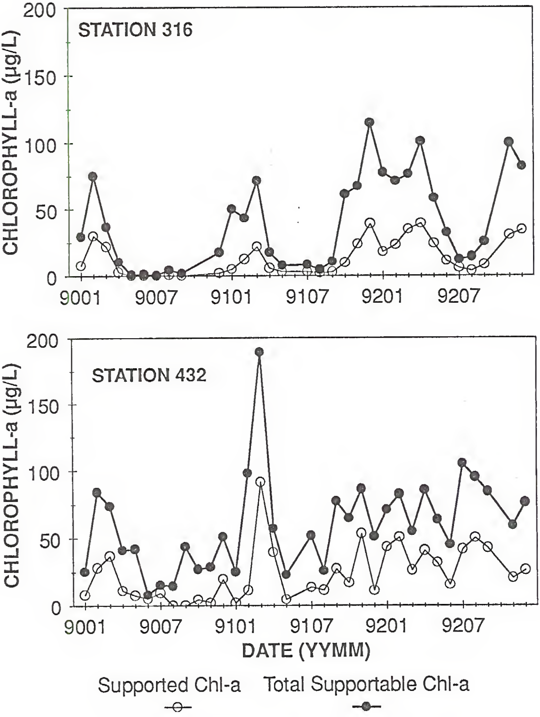


Fig. 4-8. Time series of assay derived estimates of supported chlorophyll *a* (Chl-*a*) and total Chl-*a* (initial Chl-*a* + supported Chl-*a*) for stations 316 and 432 in Lake Okeechobee.

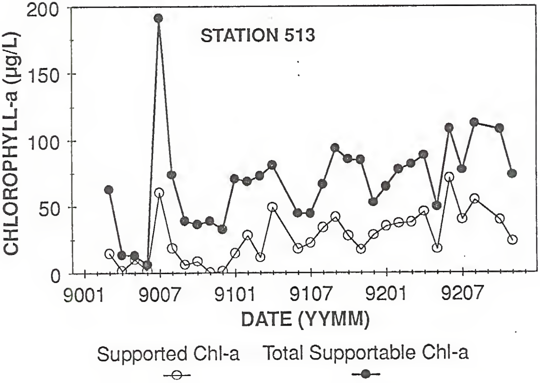


Fig. 4-9. Time series of assay derived estimates of supported chlorophyll *a* (Chl-*a*) and total Chl-*a* (inital Chl-*a* + supported Chl-*a*) for station 513 in Lake Okeechobee.

use of empirical relationships such as N:P ratios (Redfield 1958) or variants thereof, can all be confounded by non-algal constituents or by controlling factors other than N and P. The analytical techniques used for estimating concentrations of the different fractions of P (Rigler 1968, Wynne and Berman 1980) and phytoplankton biomass as Chl-*a* (Axler and Owen 1994) have been questioned as to what exactly is being measured.

Given the problems outlined above, the best approach lies in utilizing two or more independent methods of measurement. The greater the agreement in the estimates between methods the greater the confidence in the estimate. Conversely, if the estimates of surplus-P between methods varies widely the confidence in at least one of the estimates might be questioned. If the latter is the case, more detailed analysis of the data is warranted to discern possible organization in the variance between the different estimation methods. If statistical analysis can not explain the sources of variance then it must be assumed that variance is due to experimental error inherent in measurement and assay methods or to inappropriate experimental design.

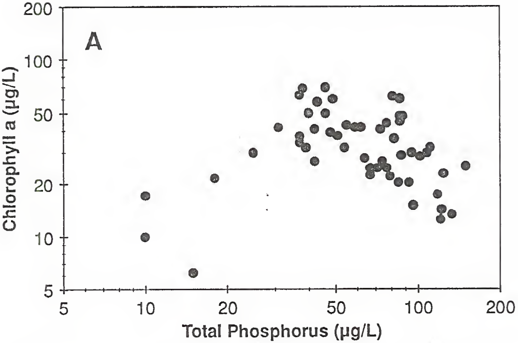
The two independent methods of assessing surplus-P, direct measurement of surplus-P and assay derived supported Chl-*a*, evaluated in this study were found to be significantly related. The slope of the regression equation: supported Chl-*a* = 17.7 + 0.909*surplus-P, is similar to the 1:1 slope for TP:Chl-*a* reported by Reynolds (1984) as the approximate average for the TP:Chl-*a* relationship found in several studies he reviewed. The 48% of the variance not explained by the regression of supported Chl-*a* on surplus-P may be attributable to non-algal contribution to the HEP component of surplus-P and to inherent error associated with the phytoplankton biomass estimates (Axler and Owens 1994). There is also the possibility that some forms of surplus-P were present to support algal growth but were not reduced by hot-water extraction to molybdate reactive forms. Wynne and Berman (1980) and Newman et al. (1994) have shown some fraction of surplus-P is not molybdate reactive after hot-water extraction. Although the regression only explains 52% of the variance, it does show that supported Chl-*a* can be predicted from chemical measurement of surplus-P.

Eutrophic and hypereutrophic lakes, such as Lake Okeechobee, characteristically have lower Chl-*a* concentrations than would be predicted by the TP/Chl-*a* relationships shown in Fig. 4-1A. Intuitively, the TP/Chl-*a* relationship would be expected to be most significant when P is limiting and most or all the TP is contained in the phytoplankton in constitutive forms. However, this relationship is biased if, as shown here, surplus-P accounts for as much as half the TP. In this case, measurement of TP overestimates the P used to produce phytoplankton biomass by a factor of two. Although TP is used in the P/Chl-*a* relationship, mainly due to the availability of data, it is not necessarily directly related to phytoplankton biomass.

Measuring maximum response has been used in different forms to refine the P/Chl-*a* relationship. A common adaptation of maximum response strategy, used for temperate lakes, relates spring SRP maxima with summer Chl-*a* maxima (Sakamoto 1966). In this case, almost the entire annual supply of the P, potentially available to phytoplankton, is supplied to phytoplankton as SRP at spring turnover. The phytoplankton biomass does not immediately increase to reflect the spring increase in SRP, but rather lags as a result of temperature controlled growth rates and other factors. By summer, the Chl-*a* maximum develops in response to SRP availability. The use of the spring SRP maxima and the summer Chl-*a* maxima accounts for the time lag between the supply of the independent variable, SRP, and the response of the dependent variable, Chl-*a*. In polymictic lakes, such as Lake Okeechobee where the pattern of seasonal maxima is not as clear, the spring SRP to summer Chl-*a* relationship is not applicable.

More recent applications of the maximum response approach are the statistical analysis of outlier values (Karydis 1994) or models for conditional maxima of responses (Kaiser et al. in press) which statistically isolate interference by focusing on extreme response values. The results presented in this chapter provide experimental evidence that supports this use of maximum responses. The total Chl-*a* concentration determined at forced P-limitation is the experimentally derived equivalent of the Chl-*a* maxima utilized in the statistical approaches. A comparison of plots for assay derived total Chl-*a* X TP, for the data from the five stations in Lake Okeechobee, and initial Chl-*a* X TP (Fig. 4-10) show that using total Chl-*a* reduces some randomness of the TP/Chl-*a* relationship. Some refinements to improve the total Chl-*a* X TP fit could probably be made by subtracting out the soluble non-molybdate reactive P concentration, measured as total soluble P minus SRP, from the TP concentration and by correcting for TP associated with tripton.

The results of this study indicate that surplus-P was probably present most of the time in the north and central areas of Lake Okeechobee and seasonally prevalent in the south and southwest of the lake in all but the summer months. This is consistent with the findings that N was the primary nutrient limiting phytoplankton biomass during the summer when N and P co-limitation was in the south and southwest part of the lake (Chapters 2 and 3). Phlips et al. (In Press-a) related the N and P co-limitation found for the summer months in the southwest areas of the lake to competition for nutrients by macrophyte vegetation and



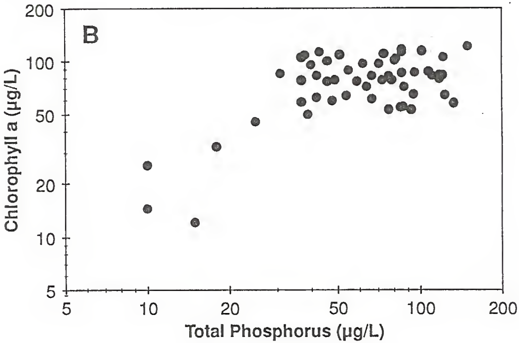


Fig. 4-10. 1992 data for chlorophyll *a* (Chl-*a*)and total phosphorus (TP) from five stations in Lake Okeechobee. Plot A is the ambient Chl-*a* X TP concentrations at the time of sampling. Plot B is the total Chl-*a* (initial + support Chl-*a*) X ambient TP.

associated epiphitic community. The surplus-P and supported Chl-*a* minima found during the summers at stations 316 and 432 support that conclusion.

The estimates for surplus-P, supported Chl-*a* and total Chl-*a* showed similar patterns between stations as those reported in the previous chapters for limiting nutrient distribution (Table 2-2). The north to south gradient observed for surplus-P, supported Chl-*a* and total Chl-*a* is consistent with similar trends found in many physical and chemical variables (Schelske 1989, Phlips et al. In Press-b). The distribution of variances for surplus P, supported Chl-*a* and total Chl-*a* by station were also similar to variances for TP, Chl-*a*, mean light availability reported by Phlips et al. (1993a) for the same stations in Lake Okeechobee.

This chapter focused on developing quantitative methods to estimate surplus phosphorus in a eutrophic system, Lake Okeechobee. The results indicate there is significant agreement between two independent methods, direct measurement of surplus-P and NEB derived supported Chl-*a*. Additional refinements should be evaluated to increase precision including measurements of other forms of P, particularly non-available mineral P, apatite and non-available soluble P. Quantifying surplus-P by a routine chemical analysis should be a valuable tool for developing and evaluating aquatic resource management goals. It can be used to determine what fraction of TP is available but not utilized for production of phytoplankton biomass. This fraction of TP can be related to the quantity of P reduction necessary to reach a P-limited condition. Reduction of P below this level can then be assumed to result in reduced phytoplankton biomass.

CHAPTER 5
SUMMARY

The research presented in this dissertation uses the nutrient enrichment bioassay (NEB) to gain new insights in the dynamics of phytoplankton ecology in Lake Okeechobee, Florida. The research was divided into three sequential phases: 1) development of NEB analysis methods and frequency analysis of the distribution of limiting nutrient classifications, 2) use of discriminant function analysis to predict and map the distribution of limiting nutrient classifications and 3) development of a quantitative analysis using NEB results to estimate the surplus phosphorus (surplus-P) or unutilized phosphorus (P) potential for phytoplankton biomass found in different regions of Lake Okeechobee over a three-year period.

The results showed the phytoplankton community in Lake Okeechobee to be predominantly nitrogen (N) limited. The second most prevalent NEB response was growth in the control treatment which indicated the phytoplankton were not limited by N or P. This indicated some other factor or factors increased phytoplankton standing crop. Phosphorus was never a primary limiting factor although N and P co-limitation was found. Frequency analysis of limiting nutrient classifications showed both spatial and temporal organization to the type and frequency of limiting factors. Lake Okeechobee, therefore, is not a homogeneous mixed reactor and thus efforts to study or predict phytoplankton community responses in the lake should be based on data which includes the spatial and temporal heterogeneity of limiting factors.

Limiting nutrient classifications from the NEB were matched with physical and chemical data from each sample date to produce a calibration data set for discriminant function analysis. Discriminant functions were defined for N, N and P co-limited and not N or P limited conditions and used to predict a limiting nutrient classification for each observation of a physical and chemical data set of 6469 observations collected from 76 stations over a three-year period. Nitrogen was the primary limiting factor from the lake shore to three-m depth contour for all seasons. Neither N nor P was limiting in areas deeper than three meters for all but the summer months when N was limiting. Water column depth, substrate type and season were related to the temporal and spatial patterns of limiting nutrient classifications in determining when N was the limiting nutrient.

Nutrient enrichment bioassays were used to determine the phytoplankton biomass, estimated as chlorophyll *a* (Chl-*a*), that could be supported by surplus P (surplus-P). Surplus-P was defined to be all forms of P that can be directly utilized by phytoplankton to support growth. In each assay, the primary limiting factor was supplied in sufficient quantity to stimulate phytoplankton growth until secondary P limitation was attained. The difference between the initial Chl-*a* concentration and the Chl-*a* concentration attained at secondary P limitation was defined as the supported Chl-*a*. Surplus-P was also estimated directly from the sum of the chemical analysis of soluble molybdate reactive P (SRP) and hot-water extractable polyphosphates (HEP). There was a significant linear relationship between surplus-P and supported Chl-*a*. The supported Chl-*a* and surplus-P for the 5 stations averaged 31 µg/L and 32 µg/L respectively with *C.V.*s of 79% and 74%. Supported Chl-*a* comprised approximately 50% of total Chl-*a* and surplus-P comprised over 50% of TP. I concluded that, as a general overview, 50% of the P available to support phytoplankton biomass was not utilized by phytoplankton and that this surplus-P comprised approximately 50% of the TP in the system.

Spatial and seasonal differences in supported Chl-*a* concentration were found. Seasonal influences were most evident at the south and southwest stations where low supported Chl-*a* in the summer months corresponded with abundance of macrophytes that probably competed for nutrients with phytoplankton communities. The three north and central stations showed no discernable seasonal periodicity and were probably most strongly influenced by stochastic events and lake stage. A spatial gradient of supported Chl-*a* was found, with the highest levels at the north lake station and lowest at the southern lake station.

This research has shown that the NEB can be a powerful tool for experimentally testing critical assumptions of empirical relationships between nutrients and algal biomass and for complementing ecological studies. Specifically, the primary assumption of P limitation in the TP/Chl-*a* relationship can be tested. In lakes where there is no relationship of Chl-*a* to TP, the collection of additional information about different P fractions is indicated. In the case of Lake Okeechobee, the use of TP concentration alone was shown to be inadequate to predict phytoplankton biomass. I have shown that P is not the primary limiting nutrient and approximately 50% of the TP in Lake Okeechobee is not expressed in phytoplankton biomass. Measurement of SRP and HEP provides the data from which the surplus fraction of TP can be estimated.

Quantifying the surplus-P in a lake provides a direct estimate of the amount of P that must be reduced before phytoplankton standing crop will be nutrient limited by P. This direct estimate of surplus-P can then be used to confirm empirically derived estimates of P that must be removed to meet management goals. Once a goal for P removal is established then the feasibility of achieving that goal can be evaluated. These analytical approaches should be applicable in estimating surplus-P over the entire range of aquatic ecosystems. This research points out new approaches that will contribute to a better understanding and prediction of phytoplankton-nutrient relationships.

REFERENCES

Aldridge, F.J., Schelske, C.L. & Carrick, H. (1993): Nutrient limitation in a hypereutrophic Florida lake. - Arch. Hydrobiol. 127(1):21-37.

APHA (1989): Standard methods for the examination of water and wastewater, 17th ed. - American Public Health Association, Washington, D.C.

Aumen, N.G. (In Press): History of human-related impacts to Lake Okeechobee, Florida (U.S.A.), related research, and lake management issues. - Arch. Hydrobiol.

Axler, R.P. & Owen, C.J. (1994): Measuring chlorophyll and phaeophytin: Whom should you believe. - Lake Reserv. Managem. (8(2):143-151.

Baker, L.A., Brezonik, P.L. & Kratzer, C.R. (1985): Nutrient load models for Florida lake. In: Lakes and Reservoir Management - Practical Applications, Proceedings Fourth Annual Conference and International Symposium. October 16-19, 1984. North American Lale Management Society. P.253-258.

Berman, T. (1970): Alkaline phosphatases and phosphorus availability in Lake Kinneret. - Limnol. Oceanogr. 15:663-74.

Brezonik, P.L. & Frederico, A. (1975): Effects of backpumping from agricultural drainage canals on water quality in Lake Okeechobee. Report no. ENV-07-75-2. - Final Report to Florida Department of Pollution Control. 64 pp.

Canfield, D.E. Jr. (1983): Prediction of chlorophyll *a* concentrations in Florida lakes: The importance of phosphorus and nitrogen. - Water Res. Bull. 19:255-62.

Canfield, D.E. Jr. & Hoyer, M.V. (1988): The eutrophication of Lake Okeechobee. - Lake and Reserv Managem. 4:91-99.

Carlson, R.E. (1977): A trophic state index for lakes. - Limnol. Oceanogr. 22:361-8.

Carper, G.L. & Bachmann, R.W. (1984): Wind resuspension of sediments in a prairie lake. - Can. J. Fish. Aquat. Sci. 41:1763-67.

Carrick, H.J., Aldridge, F.J.& Schelske, C.L. (1993): Wind influences phytoplankton biomass and composition in a shallow, productive lake. - Limnol. Oceanogr. 38(6):1179-1192.

Chrost, R.J. & Overbeck, J. (1987): Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake PluBsee (north German eutrophic lake). - Microb. Ecol. 13:229-248.

Dierberg, F.E. (1992): The littoral zone of Lake Okeechobee as a source of phosphorus after a drawdown. - Envrion. Managem. 13:729-742.

Dillon, P.J. (1974): A critical review of Vollenweider's nutrient budget model and other related models. - Water Resources 10(5):969-989.

Dillon, P.J. & Rigler, F.H. (1974): Phosphorus-chlorophyll relationships in lakes. - Limnol. Oceanogr. 19:767-73.

Dodds, W.K. & Priscu, J.C. (1990): A comparison of methods for assessment of nutrient deficiency of phytoplankton in a large oligotrophic lake. - Can. J. Fish. Aquat. Sci. 47:2328-2338.

Dokulil, M., Metz, H. & Jewson, D. (Eds.) (1979): Shallow lakes: Contributions to their limnology. - Junk Publ., Illmitz, Austria.

Elser, J.J. & Kimmel, B.L. (1986): Alteration of phytoplankton phosphorus status during enrichment experiments: Implications for interpreting nutrient enrichment results. - Hydrobiologia 133:217-222.

Elser, J.J., Marzolf, E.R. & Goldman, C.R. (1990): Phosphorus and nitrogen limitation of phytoplankton growth in freshwaters of North America: A review and critique of environmental enrichments. - Can J. Fish. Aquat. Sci., 47:1468-1477.

Fitzgerald, G.P. & Nelson, T.C. (1966): Extractive analysis for limiting or surplus phosphorus in algae. - J. Phycol. 2:32-37.

Fuhs, G.W., Demmerla, S.D., Canelli, E. & Chem, M, (1972): Characterization of phosphorus-limited plankton algae. - Special Symposia Limnol. Oceanogr. 1:113-133.

Ganf, G.G. (1974): Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). - J. Ecology 62:611-629.

Ganf, G.G. & Viner, A.B. (1973): Ecological stability in a shallow equatorial lake (Lake George, Uganda). - Proc. R. Soc. Lond. B. 184:321-346.

Gibson, E.E. (1971): Nutrient limitation. - J. Water Pollution Control Fed. 43(12):2436-2440.

Havens, K.E. (1994): Seasonal and spatial variation in nutrient limitation in a shallow sub-tropical lake (Lake Okeechobee, Florida) as evidenced by trophic state index deviations. - Arch. Hydrobiol. 131(1):39-53.

Healey, F.P. & Hendzel, L.L. (1980): Physiological indicators of nutrient deficiency in lake phytoplankton. - Can. J. Fish. Aquat. Sci. 37:442-453.

Henry, R., Hino, K., Tundisi, J.G. & Ribeiro, J.S.B. (1985): Responses of phytoplankton in Lake Jacaretinga to enrichment with nitrogen and phosphorus in concentrations similar to those of the River Solimoes (Amazon, Brazil). - Arch. Hydrobiol. 130(4):453-477.

Howarth, R.W. (1988): Nutrient limitation of net primary production in marine ecosystems. - Ann. Rev. Ecol. 19:89-110.

Hoyer, M.V. & Jones, J.R. (1983): Factors affecting the relation between phosphorus and chlorophyll a in midwestern reservoirs. - Can. J. Fish. Aquatic. Sci. 40:192-199.

Jansson, M., Olsson, H. & Pettersson, K. (1988): Phosphatases: origin, characteristics and function in lakes. - Hydrobiol. 170:157-175.

Janus, L.L., Soballe, D.M. & Jones, B.L. (1990): Nutrient budget analysis and phosphorus loading goal for Lake Okeechobee, Florida. - Verh. Internat. Verein. Limnol. 24:538-546.

Janus, L.L. & Vollenweider, R.A. (1981): The OECD Cooperative Programme on Eutrophication: Canadian Contribution. IWD, Burlington, Ontario. - NWRI Scientific Series No. 131, 450p.

Jones, J.R. & Bachmann, R.W. (1976): Prediction of phosphorus and chlorophyll level in lakes. - J. Water Poll. Control Fed. 48:2176-82.

Kaiser, M.S., Speckman, P.L. & Jones, J.R. (In Press): Statistical models for limiting nutrient relations in inland waters. - Jour. Amer. Statistical Assoc.

Karydis, M. (1994): Environmental quality assessment based on analysis of extreme values: A practical approach for evaluating eutrophication. - J. Environ. Sci. Health. A29(4):775-791.

Kratzer, C.R. & Brezonik, P.L. (1984): Application of nutrient load models to the analysis of trophic conditions in Lake Okeechobee, Florida. - Environ. Management 8:109-120.

Lake Okeechobee Technical Advisory Committee (1986): Final report Lake Okeechobee Technical Advisory Committee. - Florida Dept. Environmental Regulation, Tallahassee, Florida.

Lean, D.R.S. & White, E. (1983): Chemical and radiotracer measurements of phosphorus uptake by lake plankton. - Can. J. Fish. Aquat. Sci. 4:147-155.

Lewis, W.M. (1973): The thermal regime of Lake Lanao (Philippines) and its theoretical implications for tropical lakes. - Limnol. Oceanogr. 18:200-217.

Lorenzen, C.J. (1966): A method for the continuous measurement of *in vivo* chlorophyll concentration. - Deep Sea Research 13:223-7.

Lund, J.W.G. & Reynolds, C.S. (1982): The development and operation of large limnetic enclosures in Blelham Tarn, English Lake District, and their contribution to phytoplankton ecology. - Progress in Phycological Research 1:1-65.

Maceina, M.J. & Soballe, D.M. (1990): Wind-related limnological variation in Lake Okeechobee, Florida. - Lake Reserv. Managem. 6:93-100

MacGill, R.A., Gatewood, S.E., Hutchinson, C. & Walker, D.D. (1976): Final report on the special project to prevent eutrophication of Lake Okeechobee, Florida. - Rep. DSP-BCP-36-76. Florida Division State Planning, Tallahassee, Florida

McComb, R.B., Bowers, G.N. & Posen, S. (1979): Alkaline phosphatases. - Plenum Press, New York. 989pp.

Newman, S., Aldridge, F.J., Phlips, E.J. & Reddy, K.R. (1994): Assessment of phosphorus availability for natural phytoplankton populations from a hypereutrophic lake. - Arch. Hydrobiol. 130(4):409-427.

OECD (1982): Eutrophication of waters. Monitoring, assessment and control. Final report. - Organization for Economic Cooperation and Development, Cooperative Programme on Monitoring of Inland Waters (Eutrophication Control), Environmental Directorate, Paris. 154 pp.

Oglesby, R.T. & Schaffner, W.R. (1975): The response of lakes to phosphorus. - In: Nitrogen and Phosphorus. Food Production, Waste and the Environment, ed. K.S. Porter. Ann Arbor, Michigan, Ann Arbor Science. pp. 23-57.

Parsons, T.R., Takahashi, M. & Hargrave, B. (1984): Biological Oceanographic Processes, 3rd Edition. - Pergamon Press, New York. 330pp.

Peeters, J.C.H. & Peperzak, L. (1990): Nutrient limitation in the North Sea: A bioassay approach.- Netherlands Journal of Sea Res. 26(1):61-73.

Pettersson, K. (1980): Alkaline phospatase activity and algal surplus phosphorus as phosphorus deficiency indicators in Lake Erken. - Arch. Hydrobiol. 89:54-87.

Phlips, E.J., Aldridge, F.J. & Hanlon, C. (1994): Review of a seventeen-year chlorophyll record as it pertains to trophic status of Lake Okeechobee, Florida, USA. - Water Res. Bull. 30(2):229-238.

Phlips, E.J., Aldridge, F.J., & Hanlon, C. (In Press-a): Factors limiting phytoplankton standing crop in a shallow sub-tropical lake (Lake Okeechobee, Florida, USA). - Arch. Hydrobiol..

Phlips, E.J., Aldridge, F.J., Hansen, P., Zimba, P.V., Ihnat, J., Conroy, M. & Ritter, P. (1993): Spatial and temporal variability of trophic state parameters in a shallow subtropical lake (Lake Okeechobee, Florida, U.S.A.). - Arch. Hydrobiol. 128(4):437-458.

Phlips, E.J., Aldridge, F.J & Hanson, P. (In Press-b): Patterns of water chemistry, physical and biological parameters in a shallow subtropical lake (Lake Okeechobee, Florida, U.S.A.). - Arch. Hydrobiol..

Phlips, E.J., Aldridge, F.J., Schelske, C.L. & Crisman, T.L. (In Press-c) Relationships between light availability, chlorophyll *a* and tripton in a large shallow subtropical lake. - Limnol. Oceanogr..

Phlips, E.J. & Ihnat, J. (In Press-d): Nitrogen fixation in a shallow subtropical lake (Lake Okeechobee, Florida, USA). - Arch. Hydrobiol..

Phlips, E.J., Zimba, P.V., Hpson, M.S. & Crisman, T.L. (1993): Dynamics of plankton community in submerged plant dominated regions of Lake Okeechobee, Florida, U.S.A.. - Verh. Internat. Verein. Limnol. 25:423-426.

Reddy, K.R., Brenner, M., Fisher, M.M. & Ivanoff, D.B. (1991): Physico-chemical properties of Lake Okeechobee sediments. - In: K.R. Reddy (Ed.), Lake Okeechobee phosphorus dynamics study: Biogeochemical processes. - A Final Report to the South Florida Water Management District. 235 pp.

Redfield, A.C. (1958): The biological control of chemical factors in the environment. - Am. Sci. 46:205-222.

Reynolds, C.S. & Butterwick, C. (1979): Algal bioassays of unfertilized and artificially fertilized lake water, maintained in Lund Tubes. - Arch. Hydrobiol. Supplement, 56:166-506.

Rhee, G.Y. (1972): Competition between an alga and an aquatic bacterium for phosphate. - Limnol. Oceanogr. 17:505-514.

Rhee, G.Y. (1973): A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. - J. Phycol. 9:495-506.

Rhee, G.Y. (1974): Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. - J. Phycol. 1:470-475.

Riegman, R.F., Colijn, F., Malschaert, J.F.P., Kloosterhuis H.T. & Cadee, G.C. (1990): Assessment of growth rate limiting nutrients in the North Sea by the use of nutrient-uptake kinetics. - Neth. J. Sea Res. 26:61-73.

Rigler, F.H. (1956): A tracer study of phosphorus cycle in lake water. - Ecology. 37:550-62.

Rigler, F.H. (1968): Further observations inconsistent with the hypothesis that molybdenum blue method measures orthophosphate in lakewater. - Limnol. Oceanogr. 13:7-13.

Ryther, H.J. & Dunstan, W.M. (1971): Nitrogen, phosphorus, and eutrophication in the coastal marine environment. - Science 171:1008-1113.

Sakamoto, M. (1966): Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. - Arch. Hydrobiol. 62:1-28.

SAS (1989): SAS statistical program for personal computers, version 6.04. - SAS Institute, Cary, North Carolina.

Schelske, C.L. (1989): Assessment of nutrient effects and nutrient limitation in Lake Okeechobee. - Water Resources Bull. 25(6):1119-1130.

Schelske, C.L. (1984): *In situ* and natural phytoplankton assemblage bioassays. - In: L.E. Shubert (ed.). Algae as ecological indicators. - Academic Press. London. pp.15-47.

Schelske, C.L., Rothman, E.D. & Simmons, M.S. (1978): Comparison of bioassay procedures for growth-limiting nutrients in the Laurentian Great Lakes. - Mitt. Internat. Verein. Limnol. 21:65-80.

Schelske, C.L., Rothman, E.D., Stoermer, E.F. & Santiago, M.A. (1974): Responses to phosphorus limited Lake Michigan phytoplankton to factorial enrichments with nitrogen and phosphorus. - Limnol. Oceanogr. 19:409-419.

Schindler, D.W. (1977): Evolution of phosphorus limitation in lakes. - Science 195:260-262.

Smith, R.E.H. & Kalff, J. (1981): The effects of phosphorus limitation on algal growth rates: evidence from alkaline phosphatase. - Can. J. Fish. Aquat. Sci. 38:1421-27.

Sommer, U. (1989): Nutrient status and nutrient competition of phytoplankton in a shallow, hypereutrophic lake. - Limnol. Oceanogr. 34(7):1162-1173.

Stefan, H.T., Skoglund, T. & Megard, R.O. (1976): Wind control of algal growth in eutrophic lakes. - Amer. Soc. Civil Engr. Div. 102:1201-13.

Stewart, W.D.P., Pemble, M. & Al-Ugaily, L. (1978): Nitrogen and phosphorus storage and utilization in blue-green algae. - Mitt. Internat. Verein. Limnol. 21:224-247.

Thornton, J.A. (1987): Aspects of eutrophication management in tropical/sub-tropical regions. - J. Limnol. Soc. So. Afr. 13(1)25-43.

U.S. Environmental Protection Agency (1971): Algal assay procedure, bottle test. - National Eutrophication Research Program, Corvallis, Oregon.

Vincent, W.F., Wurstbaugh, W., Vincent, C.L. & Richerson, P.J. (1984): Seasonal dynamics of nutrient limitation in a tropical high-altitude lake (Lake Titicaca, Peru-Bolivia): Application of physiological bioassays. - Limnol. Oceanogr. 29:540-552.

Vollenweider, R.A. (1975): Input-output models with special reference to phosphorus loading concept in limnology. - Schweiz. Zeit. Hydrol. 37:53-83.

Vollenweider, R.A. & Kerekes, J.J. (1982): The Monitoring of Inland Waters: Eutrophication Control. Conclusions of the OECD Cooperative Programme on Eutrophication. - OECD Secretariat, Environment Directorate. Paris, France.

Wetzel, R.G. (1983): Limnology Second Edition. - Saunders, Philadelphia, PA. 767pp.

Wynne, D. & Berman, T. (1980): Hotwater extractable phosphorus - an indicator of nutritional status of *Peridinium cinctum* (Dinophyceae) from Lake Kinneret (Israel). - J. Phycol. 16:40-46.

BIOGRAPHICAL SKETCH

Frederick J. Aldridge was born October 24, 1954, in Lake Charles, Louisiana. He attended public and private schools in Little Rock, Arkansas, where he graduated from Little Rock Catholic High School in 1972. He attended the University of Arkansas at Fayetteville his freshman year then transferred to Texas A&M University in College Station, Texas, where he received his Bachelor of Science in fisheries ecology in December, 1976. He served two years in Guatemala, Central America, as a Peace Corps volunteer working with the Ministry of Agriculture to evaluate aquaculture potential in that country. From May to December, 1980, he participated in an invitational aquaculture program in Israel. He worked as a biological scientist in the Department of Fisheries and Aquatic Sciences at the University of Florida from 1981 to 1994 and received a promotion to senior biologist in the fall of 1994. In 1986 he began a graduate study program in the Department of Fisheries and Aquatic Sciences, completing a Master of Science degree in December, 1989. In 1993 he received the Graduate Student of the Year award from the Department of Fisheries and Aquatic Sciences. He began a Ph.D. program in 1990 and was awarded his Ph.D. in fisheries and aquatic Sciences in December, 1994.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Claire L Schelske

Claire L. Schelske, Chair
Carl S. Swisher Eminent Scholar
in Water Resources

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward J. Phlips, Cochair
Associate Professor of Fisheries
and Aquatic Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Daniel E. Canfield Jr.

Daniel E. Canfield Jr.
Professor of Fisheries
and Aquatic Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank G. Nordlie
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Konda R. Reddy
Graduate Research Professor of Soil
and Water Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1994

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School